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Enteric Virus Removal in Wastewater Treatment Lagoon Systems

(U.S.) Army Medical Bioengineering Research and Development Lab., Fort Detrick, MD

Prepared for

Health Effects Research Lab.
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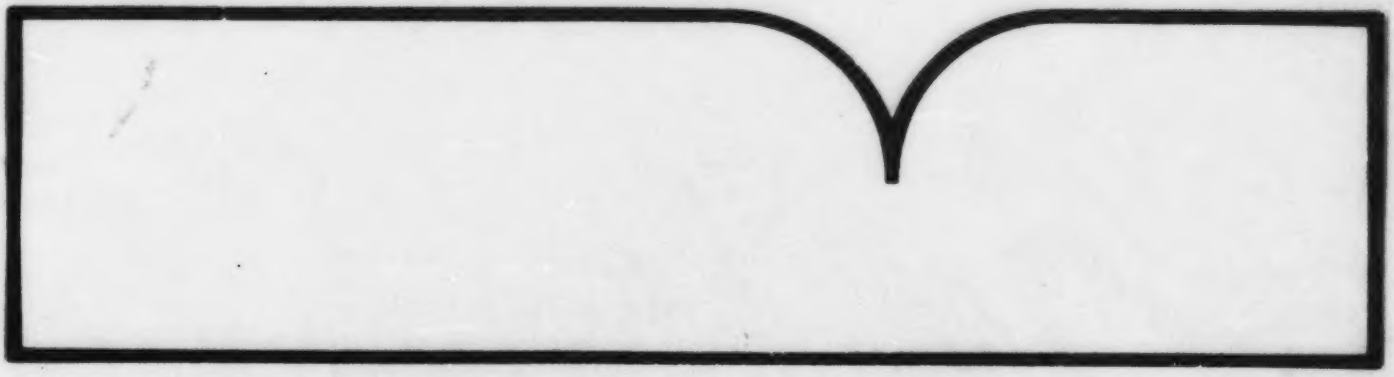

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ENTERIC VIRUS REMOVAL IN WASTEWATER TREATMENT LAGOON SYSTEMS

by

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IAG 79-D-X0728

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This study was conducted in cooperation with the US Army Corps of Engineers, Cold Regions Research and Engineering Laboratory, Hanover, NH 03755.

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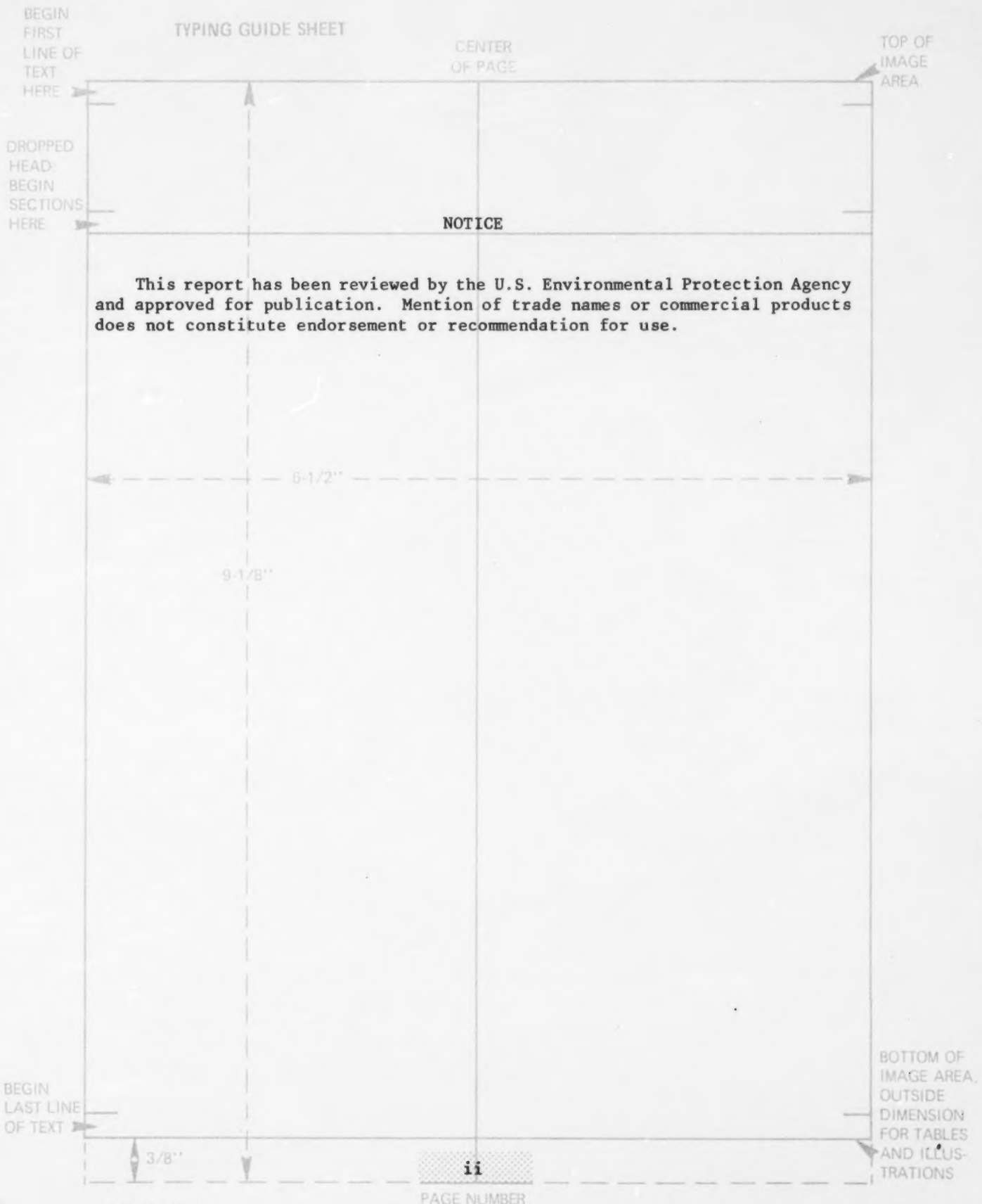

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The complexities of environmental problems originate in the deep interdependent relationships between the various physical and biological segments of man's natural and social world. Solutions to these environmental problems require an integrated program of research and development using input from a number of disciplines. The Health Effects Research Laboratory, Research Triangle Park, NC and Cincinnati, OH conducts a coordinated environmental health research program in toxicology, epidemiology and clinical studies using human volunteer subjects. Wide ranges of pollutants known or suspected to cause health problems are studied. The research focuses on air pollutants, water pollutants, toxic substances, hazardous wastes, pesticides and nonionizing radiation. The laboratory participates in the development and revision of air and water quality criteria and health assessment documents on pollutants for which regulatory actions are being considered. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of environmental regulatory decisions involving the protection of the health and welfare of all U.S. inhabitants.

This report presents the results of a field study to determine the level of viruses in effluents of lagoon wastewater treatment systems operated under various meteorological conditions. A knowledge of the pathogen content of effluents from this relatively simple and inexpensive treatment system is important in detemining the safe disposal of the effluents whether it be to soil or a watercourse.

F. Gordon Hueter, Ph.D.
Director
Health Effects Research Laboratory

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ABSTRACT

Indigenous enteric virus removal from raw wastewater was examined in facultative and partially aerated treatment lagoon systems at paired sites in the southwest, southeast, and north central regions of the U.S. Virus studies on facultative treatment lagoon systems were conducted during summer, winter and spring with sampling performed at all sites simultaneously. The virus samples were concentrated from large wastewater volumes using the bentonite adsorption-elution technique. Samples were collected from the influent wastewater and the effluent from the first and second pond in series in each system. The sampling periods were staged to approximate the residence time of the wastewater in the lagoons so that in addition to same day sample analyses estimates of virus removal through the systems could also be related hydraulically to a common body of wastewater. Fecal coliform and chemical/physical wastewater quality measurements were performed at these sites concurrently. A similar sampling regime was used for systems in which the first one or two cells receive partial mechanical aeration, though sampling was conducted only during the summer season. The indigenous virus levels in sample concentrates were measured by plaque assay on BGM, HeLa and RD cell lines.

In facultative lagoon systems, virus levels were reduced significantly throughout the year. In summer, overall virus removal in the first two ponds generally exceeded 99 percent. Virus reductions of over 95 percent were observed as combined season averages. In all seasons, the greatest contribution to virus removal occurred in the first pond of each system. There was a statistically significant difference in virus removal on the basis of season ($P < .05$), with reduced virus treatability during winter. However, because virus levels in the raw wastewater were lower in winter, the amount of virus leaving the lagoons was fairly constant throughout the year. Fecal coliform removals far exceeded virus reductions and removal of other wastewater constituents. Coliforms, TOC and suspended solids were, like virus, removed primarily in the first pond of the systems. However, statistical analysis did not reveal any wastewater parameters that were highly correlated with virus removal.

In partially aerated lagoon systems, effluent virus levels during the summer test period were comparable to those in the facultative systems. Also, in the aerated systems the greatest removal occurred in the second, or non-aerated, part of the system, characterized by longer retention times.

This report was submitted in fulfillment of Interagency Agreement IAG 79-D-X0728 between the US Army Medical Bioengineering Research and Development Laboratory and the US Environmental Protection Agency. The report covers the period June 1978 to September 1980, and work was completed as of September 1980.

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LIST OF ABBREVIATIONS

ANOVA	-- analysis of variance
BGM	-- buffalo green monkey kidney continuous cell line
BOD	-- biochemical oxygen demand
DO	-- dissolved oxygen
mg/L	-- milligrams per liter
µmho	-- micromhos, a unit of electrical conductivity
PFU	-- plaque-forming unit
PFU/L	-- PFU per liter
P-value	-- probability value
RD	-- human rhabdosarcoma continuous cell line
SS	-- suspended solids
TDS	-- total dissolved solids
TOC	-- total organic carbon

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A special thanks to Mr. Gordon W. Taylor of the USAMBRDL for his diligence in supplying the project with the large quantities of tissue culture required for the successful analysis of the indigenous virus samples.

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16. ABSTRACT

Indigenous enteric virus removal from raw wastewater was examined in facultative and partially aerated treatment lagoon systems at paired sites in the southwest, southeast, and north central regions of the U.S. The virus samples were concentrated from large wastewater volume using the bentonite adsorption-elution technique and assayed by the plaque technique on BGM, HeLa and RD cells. Temporally related samples were collected from the influent wastewater and the effluent from the first and second pond in series in each system.

In facultative lagoon systems, virus reductions of over 95 percent were observed as combined season averages. In all seasons, the greatest contribution to virus removal occurred in the first pond of each system. There was a statistically significant difference ($P < .05$) in virus removal during winter. However, virus levels in the raw wastewater were lower in winter. Statistical analysis did not reveal any wastewater parameters that were highly correlated with virus removal.

In partially aerated lagoon systems, effluent virus levels during the summer test period were comparable to those in the facultative systems. Also, in the aerated systems the greatest removal occurred in the second, or non-aerated, part of the system, characterized by longer retention times.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
enteric viruses, enteroviruses, lagoons, coliforms, wastewater	lagoon wastewater treatment, virus coliform relationship, seasonal variation in lagoon treatment	

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SECTION 1

INTRODUCTION

The presence of human enteric viruses in the environment represents a concern to the public health community, and the removal of viruses from wastewater before discharge to receiving streams is considered desirable. Conventional secondary treatment of sewage, even with terminal chlorination, does not reliably reduce the virus level released to the environment by more than 90 to 99 percent of that found in raw wastewater (1-5). While most larger communities provide secondary or tertiary wastewater treatment, other communities, especially small towns, provide less sophisticated treatment such as facultative or aerated treatment lagoon systems. Additionally, most land wastewater application systems require holding ponds to store effluent until the irrigation season. The dissemination of infectious viruses has also been shown to occur upon land wastewater application: through spray aerosols (6,7) and by translocation to groundwater (8,9). A number of studies (10-21) have demonstrated the enteric virus removal capabilities of various model or full-scale operating wastewater treatment (and holding) lagoon systems.

The finding that enteric virus removal can be relied upon in either treatment or holding lagoons in the various regions of the US throughout the seasons of the year would lessen the magnitude of health risks from virus discharged in treated wastewater. High virus removal efficiency by lagoon systems could also encourage use of these systems with the added benefits of reduced capital and operating costs, less sophisticated treatment processes and reduced disinfection requirements.

One objective of this study was to quantitatively assess the treatment/removal of indigenous human enteric viruses from wastewater in typical facultative (nonaerated) wastewater treatment lagoon systems. For these systems the study evaluated the effects of site, region, and season on the extent of virus removal. Efforts were also directed at measurement of other wastewater constituents to determine if their removal could be used to reliably serve as an indicator of virus removal. A second objective was to perform a range-finding study to provide an indication of enteric virus removal in lagoon systems where the first cell(s) (ponds) receive partial mechanical aeration. Because the US Environmental Protection Agency (USEPA) and US Army Corps of Engineers share or have mutual responsibilities for establishment of lagoon sites for domestic wastewater treatment and holding ponds for land application of wastewater, support for this study has come from the USEPA, Health Effects Research Laboratory and the US Army Cold Regions Research and Engineering Laboratory.

SECTION 2

CONCLUSIONS

Indigenous enteric virus reduction by typically designed and operated facultative wastewater treatment lagoon systems appeared to be at least equivalent to that reported in the literature for secondary biological waste treatment processes. Virus removal capabilities often exceeded 99 percent during summer.

Although there appears to be a significant statistical difference in virus removal on the basis of season, with reduced treatability in winter, the overall mean reduction of virus in system effluents exceeded 95 percent.

Virus removal in the facultative wastewater treatment lagoon systems was most extensive in the first pond of each system.

Five-day plaque analysis of concentrated indigenous enteric virus samples on BGM, HeLa, and RD cell lines yielded similar results at all sites in each region and for all seasons. Some minor differences in virus recovery or percent virus reduction in the pond systems were observed between cell lines but such differences were generally insignificant.

While enteric virus levels in raw wastewater were greatest in summer, virus removal was also higher, thus producing a consistent density of viruses leaving the system throughout the year. Average virus levels detected in lagoon system effluents were less than 2 PFU/L. Because estimated virus recovery efficiency was low (<50 percent) it is probable that actual virus levels in system effluents were at least twice that observed.

Fecal coliform removals far exceeded the removal of virus and other wastewater constituents measured. Statistical analysis did not reveal any wastewater parameters that were highly correlated with virus removal. Thus, no nonviral entities were identified as indicators of virus removal in such systems.

Enteric virus removal in summer in lagoon systems in which the first one or two cells (ponds) received partial mechanical aeration was comparable to that observed in facultative systems for the same period. The greatest virus removal in these systems occurred in the nonaerated portion, which was characterized by a longer retention time.

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SECTION 3

RECOMMENDATIONS

Additional studies should be conducted to assess virus removal in both facultative and aerated wastewater treatment lagoon systems to:

a. Validate the results of the present study over a broader sampling of operating lagoon systems.

b. Explore the mechanisms of virus removal (such as through adsorption to settleable solids) to assess the potential for enhanced virus reduction through modified design or operation of lagoons.

c. Evaluate virus removal during "worst case" environmental conditions, i.e., spring or fall turnover, extreme wind action and mixing, storm water input, effects of toxic chemicals or extreme wastewater loadings.

d. Further examine the correlation of virus removal to the treatability of other wastewater constituents in lagoons in order to establish a chemical/physical parameter monitoring method to assess virus concentrations in pond effluents.

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SECTION 4

MATERIALS AND METHODS

POND SITES STUDIED

The selection of wastewater lagoon (pond) systems to be included in the study was preceded by identification of criteria for acceptable lagoon design and performance for systems with and without mechanical aeration and for various climatic regions within the United States. U.S. Environmental Protection Agency (USEPA) Process Design Manuals, state wastewater treatment regulations and communication with persons knowledgeable in wastewater treatment lagoon systems served as sources of information. Performance criteria are included in TABLE 1 for systems without mechanical aeration and in TABLE 2 for partially aerated systems. Also included are additional selection criteria, as developed jointly by personnel of the US Enviromental Protection Agency, US Army Corps of Engineers and US Army Medical Bioengineering Research and Development Laboratory (USAMBRDL). Six sites without mechanical aeration were selected for study. These consisted of two paired sites in each of the three regions: north central, southeast and arid southwest. To the extent possible, systems similar in design, operation, size and wastewater characteristics were chosen. Two partially aerated pond systems of considerably greater wastewater loading were also included in the study for virus treatability comparisons. These were located in the upper midwest and Colorado Rocky Mountains. In each the first one or two ponds in series receive partial mechanical aeration and are of greater depth. The remaining ponds are not aerated and are characterized by longer retention times.

Pond systems were chosen after extensive communication with persons representing the USEPA, other US Government agencies, universities and especially the water resources or environmental protection agencies of various states. Each candidate system was reviewed from a historical operations standpoint with local municipal officials and with representatives of the engineering firm responsible for its construction and/or maintenance before final selection.

Characteristics of the sites chosen are shown in TABLE 3. Communities without significant industrial discharge were selected, with the exception of Muskegon, where about 60 per cent of the wastewater is of industrial origin, largely from paper mills.

All systems receive raw sewage without prior settling or other pretreatment. The systems at Muskegon and El Paso serve substantially larger populations than the other systems.

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TABLE 1. SELECTION CRITERIA FOR POND SYSTEMS LACKING MECHANICAL AERATION

Criterion	Desired Range	Acceptable Range
Population served	1,000 - 5,000	500 - 20,000
Nature of sewage	All domestic	Domestic with or without minor industry
Treatment prior to lagooning	None	None or settling pond
Number of cells in series	<2	<2
Depth of first cell		
North	4-7 ft (1.2-2.1 m)	4-7 ft (1.2-2.1 m)
South	3-6 ft (0.9-1.8 m)	3-6 ft (0.9-1.8 m)
Retention time each cell		
North	30-50 days	25-80 days
South	20-40 days	10-50 days
Discharge	Continuous	Continuous
BOD_5(lb)/acre (kg/ha)*	North: 30-50 (34-56) South: 40-60 (45-67)	20-120 (22-134) 20-180 (22-202)
Effluent quality, BOD_5	<30 mg/L	<30 mg/L
Effluent quality, suspended solids	<90 mg/L	<90 mg/L
Seepage	0-0.65 cm/day	0-0.65 cm/day
Length of service	>5 yr	>5 yr
Region/climate	Upper midwest - 2 sites Southeast - 2 sites Arid southwest - 2 sites	

* One kg/ha/day = 0.89 lb/acre/day.

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TABLE 2. SELECTION CRITERIA FOR POND SYSTEMS USING MECHANICAL AERATION

Criterion	Desired Range	Acceptable Range
Population served	1,000-20,000	1,000-150,000
Nature of sewage	All domestic	Domestic, with or without minor industry
Treatment prior to lagooning	None	None, or settling pond
Number of cells	>2	>2
Depth of first cell	10-20 ft (3.1-6.2m)	7-25 ft (2.1-7.6 m)
Retention time, aerated pond	1-3 days	1-5 days
Retention time, total	20-180 days	20-180 days
Discharge	Progressive drawdown over growing season	Same
BOD_5, aerated pond, lb/acre (kg/ha)	1,000-5,000 (1,120-5,600)	800-7,000 900-7,840
Effluent quality, BOD	<30 mg/L	< 30 mg/L
Effluent quality, suspended solids	<90 mg/L	<90 mg/L
Length of service	>5 yr	>5yr
Aeration; first cell second cell	Partial None	Partial None
Region/climate	Muskegon, MI Plus one site in front range province	

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TABLE 3. SITE CHARACTERISTICS

Region / Site	Nonaerated, Paired Sites						Sites Using Aeration	
	Upper Plains		Southeast		Arid Southwest			
	Beresford SD	Lennox SD	Shelby MS	Jonestown MS	El Paso TX	Kermit TX	Muskegon MI	Castle Rock CO
Location within State	SE	SE	NW	NW	W	W	W Central	Central
Elevation (m)	365	365	46	46	1,130	854	131	1,890
Rainfall (cm/yr)	50	50	130	130	20	28	76	43
Ice cover (mo)	2 1/2-4	2 1/2-4	None	None	None	None	3	4
Population served	3,000	1,900	3,000	1,200	55,000	8,000	150,000	3,500
Nature of sewage	Dom.	Dom.	Dom.	Dom.	Dom.	Dom.	Dom.; paper mill	Dom.
Volume treated m^3/day*	1,140	760-1,140	1,140	450	23,000	3,000	110,000	1,500

* 1,000 m^3/day = 0.264 mgd.

Design and operational characteristics of the lagoon systems comprise TABLE 4 and their loading rates, TABLE 5. In every case, final effluent quality regularly meets discharge standards for BOD and total suspended solids. Seepage from the lagoons, expressed as a daily rate, is low due to natural or artificial clay barriers. Nevertheless, in the storage lagoons at Muskegon, because of their long retention time (TABLE 5), this amounts to about 40 percent of input water. All systems have been in service for 5 years or longer, and all have at least two ponds operated in series. Calculated residence times vary considerably and are somewhat lower in the Texas systems. The six paired systems are operated in a continuous discharge mode. At Muskegon and Castle Rock, the final ponds are subject to seasonal drawdown with the water applied to cropland. Discharge from the final pond at Castle Rock is however, year-round.

Additional data were obtained on each system including influent volumes, effluent quality, and meteorological information, the last supplied by the U.S. Weather Bureau.

FIELD SAMPLING

Field sampling, virus concentration and shipment of samples to USAMBRDL were performed by local licensed environmental service contractors. These contractors also performed most physical, chemical and coliform determinations. At the initiation of the study each site was visited by USAMBRDL personnel, and the sampling contractor personnel were directly instructed in the use of the virus concentrating equipment and in sampling procedures.

The six paired sites without mechanical aeration were studied in each of the three seasonal test periods: late summer-fall, late July to October 1979; winter, January to March 1980; and spring, late March to May 1980. During each season samples were taken at least three periods in time. This sampling was nearly simultaneous at the six sites. These sampling times were on approximately 25 day intervals, thereby spanning a period of at least 50 days in each season.

The two systems using mechanical aeration were studied at only three points in time, representing approximately the beginning, midpoint and end of the summer drawdown season. Sampling was approximately simultaneous at the two sites.

On each sampling day, samples for virus concentration, virus analysis, physical and chemical parameters and coliform determinations were obtained at three points in each lagoon system. In nonaerated systems these were: (1) the influent wastewater (raw sewage) before the first lagoon; (2) the effluent from the first lagoon, or the first pair of lagoons if operated in parallel (Lennox and El Paso); and (3) the effluent from the second lagoon. In systems with mechanical aeration, sampling points were: (1) raw sewage; (2) the effluent from the mechanically aerated portion whether one pond (Castle Rock) or two in series (Muskegon); and (3) the effluent from the final pond(s), which were subjected to seasonal drawdown. At the sites with aeration, replicate samples were taken for virus concentration and evaluation.

TABLE 4. DESIGN AND PERFORMANCE CHARACTERISTICS OF POND SYSTEMS

Site	Nonaerated Paired Sites						Sites Using Partial Aeration	
	Beresford, SD	Lennox, SD	Shelby, MS	Jonestown, MS	El Paso, TX	Kermit, TX	Muskegon, MI	Castle Rock, CO
No. of ponds Operation	2 Series	3 Parallel; series 2+1	3 Series	3 Series	4 Parallel; series 2+1+1	7 Series	4 Series; parallel 1+1+2	4 Series; parallel 1+1+2
Pond size (ha)								
1st*	3.2	4.0 (2 pds)	4.9	1.6-2.0	43 (2 pds)	2.4	6.5 (2 pds)	0.3
2nd*	3.0	2.0	2.4	1.6	15	4.0	690 (2 pds)	3.8 (3 pds)
Depth (m)								
1st	1.7	1.5	1.2	1.8-2.1	1.5	2.6	3.7	3.7
2nd	1.7	1.5	1.2	1.8-2.1	1.5	2.6	1.5	1.5
Discharge	Cont.	Cont.	Cont.	Cont.	Cont.	Cont.	Growing season drawdown	Cont.; growing season
Length of service (yr)	23	17	5	5	15	25	6	10
Designer or Engr. Firm	J.T. Banner Brookings, SD	Little, Schmidt & Calda, Sioux Falls, SD	Thomas, Cleveland, MS	Jess Haley, Clarksville, MS	Parkhill, El Paso, TX	Esmond Haines, Odessa, TX		

* For system with aeration, "1st" = aerated part; 2nd = nonaerated or facultative part.

TABLE 5. LOADING CHARACTERISTICS OF POND SYSTEMS STUDIED

Site	Nonaerated, Paired Sites						Sites Using Partial Aeration	
	Beresford, SD	Lennox, SD	Shelby, MS	Jonestown, MS	El Paso, TX	Kermit, TX	Muskegon, MI	Castle Rock, CO
Population/ha								
1st*	926	469	617	659	1,270	3,290	23,150	11,090
1st & 2nd*	479	314	412	348	936	1,230	215	844
BOD_5/ha/day								
1st, or aerated	185	94	123	133	252	659	4,230	2,220
1st & 2nd	96	62	81	69	188	247	39	168
Population/1,000 m^3**								
1st	76	39	51	33	86	270	633	364
1st & 2nd	39	26	34	19	62	101	7.8	38
Calculated residence (day)								
1st	32	48	48	73	26.7	9.0	2.0	6.7
1st & 2nd	62	72	72	138	35.2	24.0	159.0	63.0

* For systems with aeration, "1st" = aerated part; "2nd" = nonaerated or facultative part.
** 1,000 m^3 = 0.81 acre-ft.

For measurement of water quality parameters other than virus, grab samples were taken from the uppermost 12 inches (30 cm) of the water column. The following were determined at each sample point per sampling day: suspended solids (SS), total organic carbon (TOC), electrical conductivity, fecal coliforms and total dissolved solids (TDS). Measurements of water temperature, pH and dissolved oxygen (DO) were made in the morning and, when possible, in the afternoon. All nonvirus analyses except TOC were performed by the contractor. TOC samples, preserved with HCl in glass-stoppered bottles, were shipped with virus concentrates under ice and analyzed at USAMBRDL using a Beckman Model 915 TOC analyzer. All wastewater quality analyses were performed according to Standard Methods (22).

VIRUS ANALYSIS

Field Concentration

For virus concentration the sample volume was 20 to 35 gal (76 to 132 L) for pond effluents and 10 to 20 gal (38 to 76 L) for raw wastewater. Pond effluent samples were simple grab samples. Raw wastewater samples were composites. The first portion was collected the previous evening while the second and third portions were collected in the early morning and at noon of the sampling day. Samples were batch-concentrated using the bentonite adsorption technique. Recovery efficiency for seeded poliovirus in various wastewaters using this method is about 40 percent (unpublished data). Preparation of each sample for filtration was as follows, with continuous stirring: (1) the pH, if above 8.0, was adjusted to pH 7.5 to 8.0 with HCl; (2) Slurried bentonite clay (laboratory grade) was added to a final concentration of 70 mg/L; (3) $CaCl_2$ was added to a final concentration of 0.01M. After 20 minutes mixing, the sample was passed through a 10-inch (25.4 cm) Johns-Manville fiberglass honeycomb high-capacity dirt-handling cartridge filter (Filterite G002, 2 mm nominal pore size), using a positive pressure water pump. Filtration was begun at 2.0 gal/min (7.6 L/min) and was discontinued when the flow rate fell below 0.5 gal/min (1.9 L/min) or if the pressure differential across the filter reached 25 psi (1.73 kg/cm^2). Each filter was placed in a sealable plastic bag and 300 mL 2 percent beef extract, pH 9.0, was added. The sealed bags were then shipped to USAMBRDL under ice. To be acceptable for virus analysis, filters were required to be received at the laboratory within 60 hr (preferably 48 hr) of the sampling time.

Concentration of the effluent virus samples in the field was also performed by the method proposed by the USEPA, Cincinnati, OH. This was done once each season at each of the six sites without mechanical aeration. An aliquot of the composited influent sample was amended with $AlCl_3$ (0.005M final concentration). It was then adjusted to pH 3.5 and stirred for 5 minutes. The sample was then passed through a Balston 100-25-C microfiber filter, 1 in x 7 in (2.5 x 17.8 cm), at 1 to 2 gal/min (3.8 to 7.6 L/min). Filtration was stopped at a pressure differential of 30 psi (2.08 kg/cm^2). Preservation and shipment of the filters was as described for the Filterite (G002) filters.

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Reconcentration of Virus Samples

Refrigerated virus concentrate samples received from the field were reconcentrated in the laboratory and frozen on the day of receipt. The beef extract eluent was brought to 600 mL and then adjusted to pH 9.0. Eluent bathing the filter was then recirculated through the filter with a peristaltic pump at 100 mL/min to achieve elution of virus. Elution was continued for 20 minutes with sonication (sonic waterbath) applied during the final 15 min. The eluent was then subjected to a coarse filtration using a 293 mm Millipore AP25 filter (pore size ~ 1 μm) to remove residual wastewater solids and bentonite.

Reconcentration was then performed using the low pH organic flocculation method of Katzenelson (21). The filtered sample eluent was adjusted to pH 3.5 and was then adjusted to a conductivity of 7.5 μmho using 3 percent NaCl. The sample was stirred for 30 min to encourage floc formation and centrifuged 15 min at 5000 rpm in a Sorvall RC2B refrigerated centrifuge using a SS35 rotor. The supernatant fluid was discarded. The pellet was resuspended in approximately 20 mL 0.15 M Na_2HPO_4 with rigorous mixing and then frozen at -70°C for a minimum of 24 hr. Final sample volume was about 20 mL for influent samples and about 25 to 30 mL for pond samples. Thus total sample concentration was about 2500-fold for influent samples and 4000-fold for pond samples.

Plating and Enumeration

Frozen reconcentrated samples were thawed in a 35°C water bath. Then 0.3-mL ethyl ether per 5-mL sample was added to reduce bacterial contamination, and 35°C incubation was continued 1 hour to dispel ether. One-tenth volume 10X Hanks' basic salt solution (Hanks' BSS) was then added, and the sample was plated on cell cultures by routine plating methods. Continuous heteroploid cell lines used for each assay were HeLa from Dr. C.A. Sorber, University of Texas, San Antonio, TX; Buffalo green monkey kidney (BGM) from Dr. G. Berg, USEPA, Cincinnati, OH; and human rhabdosarcoma (RD) from Dr. E. Lennette, State of California Health Department. In all cases 0.7 mL of undiluted reconcentrated virus sample was plated on cell monolayers grown in 100-mm tissue culture dishes. A minimum of 5.6 mL (eight 100 mm plates) were used for each sample concentrate. From pond influent samples (raw wastewater), 10^{-1} and 10^{-2} dilutions were also plated on cell culture monolayers in 60-mm tissue culture dishes with an inoculum of 0.25 mL per plate. Samples were allowed to contact the cell cultures for a 90-min adsorption period. A solid agar overlay was then added to each infected monolayer. For BGM monolayers this consisted of growth medium 199 (1X) containing 10 percent fetal calf serum and solidified with 1 percent purified agar of low electrolyte content. Gentamycin (100 μg/mL), kanamycin (100 μg/mL) and mycostatin (50 μg/mL) were also included. For HeLa and RD cell cultures, medium 199 was replaced by minimum essential medium--Eagles (MEM) (1X) growth medium. RD cells were also supplemented with 1X glutamine and MEM (1X) vitamins. After 4 days incubation at 35°C in a 5 percent CO_2 in air atmosphere, the assay cultures were fed with additional agar growth medium of the same composition at 70 percent of the original overlay volume. The cultures were also stained with neutral red on day 4 (1:2000 dilution).

Plaques were counted visually on day 4 and on each successive day until degeneration of the cell monolayer. The use of ether to control bacterial and fungal contamination was not 100 percent effective, possibly because of the presence of relatively resistant organisms or to some protection afforded organisms by the abundant organic material in the virus reconcentrates. If no plaques were found from a sample on the first assay or if bacterial/fungal contamination interfered with the assay, a second aliquot of the frozen reconcentrate was assayed in the same manner. Approximately 20 to 25 percent of the total sample concentrate volume was normally assayed for virus. Results were expressed as PFU virus/L of original sample.

Plaque Verification

Plaque verification was performed during each sample assay and for each cell line used. The mouth end of a sterile plastic disposable 10-mL pipette (7 mm) was used to recover each randomly chosen plaque (or outer perimeter of the plaque if very large), with scraping of the monolayer. The agar and cell material were placed into a tube containing 2 mL of 1X Hanks' BSS with 1 percent fetal calf serum and mixed well on a vortex mixer. The material was then passed from a syringe through a Millipore Swinix filter holder containing a 0.2-μm bacteriological filter membrane. The filtrate was stored frozen at -85°C until assay. Plaque verification was performed by dilution of the thawed (25°C) filtered plaque isolate with subsequent plating on the same cell line from which the plaque was isolated. After adsorption of virus to cells, standard agar overlay medium was added. The cultures were incubated, stained with neutral red (on day 4) and observed for plaque production for up to 9 days. Positive plaquing material was stored at -85°C for possible future identification of virus strains.

Quality Assurance

Once or twice during each seasonal study, from each of the six paired sites lacking mechanical aeration, an aliquot (<1L) of the influent composite was shipped unprocessed to USAMBRDL for virus concentration and analysis in the laboratory. Processing was performed in the same manner as field concentration except for the size and material of filter used. This served as a quality assurance sample for the overall efficiency of field concentration of raw wastewater samples.

STATISTICAL ANALYSIS

Data analysis centered on the BGM cell line, because this was the most complete data set. For each season, region, and site, the influent and effluent virus concentrations (PFU/L) were estimated for each pond using geometric mean concentrations over the 3- to 4-replicate sampling days. One replicate from the Beresford BGM cell line data was determined to have an abnormally low influent value and was removed from the data. Two replicates were removed from the RD cell line data that were not consistent with the other replicates in the series. Percentage virus reductions across pond one and across both ponds were estimated without adjusting for pond retention times. Data were also analyzed with lag times introduced into the analysis to account for pond retention times. The percentage virus reductions were tested

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using the analysis of variance (ANOVA) technique (23) for the main effects of season, region, site within region, and the interaction effect of season X region (which measures whether seasonal effects vary among regions). The arcsin transformation was used to stabilize the variances and to normalize the data. Correlations of percentage virus reduction with the physical/chemical parameters, averaged over the replicate days, were calculated within each season and across seasons. Evaluations were made of the BGM cell line results to similar analyses of the RD and HeLa cell lines to assess comparability.

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SECTION 5

RESULTS

METEOROLOGICAL DATA

Local meteorological data are given in TABLE 6 for weather stations geographically close to the respective pairs of nonaerated lagoon sites. These stations are at Sioux Falls, SD (north central), Memphis, TN (southeast), and Midland, TX (arid southwest). Monthly values are given for mean temperature, wind speed, and cloud cover (sunrise to sunset). Seasonal averages or totals are given for late summer, 1979, winter 1979-1980, and spring 1980.

As anticipated, markedly lower mean temperatures were observed at Sioux Falls than at the other stations. Precipitation was least at Midland. Seasonally, precipitation was lowest in the winter at all three stations. Mean wind speeds varied, but little difference was observed by station or season. Wind speeds were highest at Midland and lowest at Memphis. Cloud cover was least at Midland. Seasonally, cloud cover was least in the summer 1979.

Mean daily solar radiation (year-round) for U.S. Weather Bureau stations located within the geographic region represented by each study site or pair of sites was also obtained. Radiation tends to be high in the arid southwest (466 to 536 Langleys), low in the north central area (348 to 392 Langleys) and moderate in the southeast (355 to 418 Langleys). There is little difference in this regard between the north central and the southeast regions.

NONAERATED (FACULTATIVE) LAGOON SYSTEMS

Physical, Chemical, and Bacteriological Water Quality Parameters

Mean values of various water quality parameters for pond influents during each season are given in TABLE 7. Also given are overall percentage reductions for the parameters at each study site in each season. Seasonal data for individual ponds are given in the Appendix, TABLES A-1 to A-11.

Highest removal of all parameters measured was observed for fecal coliforms. The greatest contribution to total removal usually occurred in the first lagoon of each system; total removal ranged up to 5 orders of magnitude. More modest removal was observed as a trend in the winter seasons, though at the Texas sites summer fecal coliform removals were also relatively lower. Fecal coliform levels exiting from the pond systems often were <200/100 mL.

TABLE 6. MONTHLY AVERAGES OF METEOROLOGICAL MEASUREMENTS AT STATIONS CLOSE TO PAIRED STUDY SITES DURING STUDY PERIOD, 1979-1980

	Temperature (°C)			Precipitation (cm)			Wind Speed (m/sec)			Sky Cover Daytime (tenths)		
	SF*	Mem*	Midl*	SF	Mem	Midl	SF	Mem	Midl	SF	Mem	Midl
Summer-Fall												
Aug	21.1	27.2	26.1	11.0	15.0	5.5	3.9	3.0	4.8	6.0	5.2	3.9
Sep	17.9	23.0	23.9	10.2	11.4	0.20	4.0	3.4	3.9	2.2	4.0	2.9
Oct	9.0	18.8	20.1	8.4	6.4	2.5	4.5	4.4	5.2	5.8	4.2	2.7
Total				29.6	32.8	8.2						
Mean	16.0	23.0	23.4				4.1	3.6	4.6	4.7	4.5	3.2
Winter												
Dec	-2.7	7.4		0.10	12.5		4.5	4.4		6.1	4.9	
Jan	-7.7	6.2	7.2	0.46	8.2	1.2	5.9	4.4	5.0	7.4	7.6	7.4
Feb	-7.7	4.2	7.9	1.2	2.8	0.74	5.4	4.5	5.5	7.0	6.7	5.4
Total				1.8	23.5	1.9						
Mean	-6.0	5.9	7.6				5.3	4.4	5.3	6.8	6.4	6.4
Spring												
Mar	-0.2	9.7	11.3	1.8	27.6	Trace	5.5	5.0	6.4	6.7	7.3	5.0
Apr	10.1	16.1	15.1	2.0	19.1	2.2	5.2	4.5	5.9	4.7	5.1	3.3
May	14.7	22.5	20.6	6.4	11.3	4.7	4.4	3.7	5.6	5.4	6.1	4.7
Total				10.2	58.0	6.9						
Mean	8.2	16.1	15.1				5.0	4.4	6.0	5.6	6.2	4.3

* SF = Sioux Falls, SD; Mem = Memphis, TN; Midl = Midland, TX.

TABLE 7. NONAERATED SYSTEMS: WASTEWATER PHYSICAL/CHEMICAL CHARACTERISTICS AND FECAL COLIFORMS

Site and Season	TOC		Total Suspended Solids		Total Dissolved Solids		Conductivity		Fecal Coliform	
	Infl. mg/L	% Removal	Infl. mg/L	% Removal	Infl. mg/L	% Change	Infl. umho/cm	% Change	Infl. Log CFU/L	% Removal
Summer										
Jonestown, MS	84	55	165	58	460	28	595	37	7.62	99.95
Shelby, MS	149	55	143	61	751	2	948	15	6.95	99.95
El Paso, TX	109	43	168	97	845	19	750	7	6.32	98.76
Kermit, TX	100	45	149	70	666	-4	1,039	-4	6.42	99.07
Beresford, SD	77	66	102	94	1,480	-5	2,120	12	6.57	99.99
Lennox, SD	76	53	81	49	2,970	-8	3,775	1	6.82	>99.99
Winter										
Jonestown, MS	78	60	84	14	481	22	570	21	6.39	99.91
Shelby, MS	86	63	136	17	912	25	1,037	22	6.63	99.83
El Paso, TX	79	30	133	-1	723	-0	1,083	-1	6.05	>99.999
Kermit, TX	98	55	249	71	557	-13	870	-13	6.95	99.14
Beresford, SD	58	24	132	87	1,295	-12	2,030	-5	6.48	99.57
Lennox, SD	104	55	185	91	3,043	-2	3,8800	-5	6.87	97.48
Spring										
Jonestown, MS	135	84	225	69	574	46	642	44	7.04	99.97
Shelby, MS	112	75	69	13	799	31	995	37	6.82	99.98
El Paso, TX	72	20	154	10	750	-4	1,177	32	8.03	>99.999
Kermit, TX	107	55	200	7	489	-19	763	-18	6.84	99.95
Beresford, SD	64	56	146	83	1,603	11	2,357	11	6.37	>99.999
Lennox, SD	94	55	143	73	3,249	12	4,320	8	7.04	99.99

Total organic carbon removal also occurred mainly in the first lagoon of each system, but mean values ranged only from 20 to 84 percent total reduction.

Suspended solids removal was more variable, with mean values ranging from 0 to 97 percent. In winter and spring, suspended solids often increased from the first to the second pond. Consistently good reduction was observed for all systems during the summer season and at the South Dakota sites in all seasons. The contribution of algae to suspended solids was not established, but visually they were most prevalent in the first ponds. As anticipated little change was observed in values for dissolved solids and conductivity; the small changes observed were largely confined to the two southeastern sites.

Data on temperature, pH, and DO for the various influents and ponded waters are shown in TABLE 8. Full data are given in Appendix A, TABLE A-2. Mean water temperatures for the spring season were in all cases intermediate between those of the other seasons. Highest water temperatures for the spring and summer seasons occurred in Mississippi, while in winter the Texas ponds were the warmest. Only modest change in temperature was observed between AM and PM readings.

The pH of the ponds was generally above the pH of the influent wastewater, but only in the winter season was pH of the second pond usually higher than that of the first. pH values above 9 were common only at the two southeastern sites, especially in the summer-fall season. For all regions pH values tended to be higher in summer than in winter. These variations are most likely due to the effects of the algae populations in the ponds and their uptake of CO_2 during photosynthesis. Algal populations were visually greater in the south and in summer.

The DO of the influent water was quite low in all systems as anticipated. Within the lagoons the DO increased substantially except at the north central sites in winter, when they were generally under ice cover. Greatest DO was observed to be generated in the southeast, while for all regions summer values exceeded winter values. DO was often higher in the first of the two ponds, where greater algal activity was evident.

Modest changes in water temperature, pH and DO were commonly encountered between morning and afternoon readings. However, a simultaneous rise in pH and DO, an indication of high algal activity, occurred consistently only at Jonestown, Shelby and Lennox, in the summer season.

Enteric Virus Levels and Virus Reduction

To analyze the data on virus levels and percent virus reduction, two modes of comparison of influent to effluent levels were examined:

(1) Data from influent and pond effluent virus samples obtained on the same day were compared, and

TABLE 8. NONAERATED SYSTEMS: WASTEWATER CHARACTERISTICS DETERMINED TWICE/SAMPLING DAY

Site and Season	Water Temp (°C)					pH					Dissolved O2 (mg/L)				
	Infl.	Pond I		Pond II		Infl.	Pond I		Pond II		Infl.	Pond I		Pond II	
		AM	PM	AM	PM		AM	PM	AM	PM		AM	PM	AM	PM
Summer															
Jonestown, MS	26.6	30.1	32.1	30.0	31.6	7.7	8.2	9.1	7.9	8.4	1.2	6.2	10.0	4.6	9.3
Shelby, MS	28.3	29.2	32.0	28.0	30.7	7.7	9.3	10.0	9.4	10.5	0.5	9.8	13.9	8.1	13.2
El Paso, TX	28.5	28.5	28.7	28.0	28.1	7.2	8.0	8.1	8.2	8.2	0.1	1.5	1.6	2.5	2.5
Kermit, TX	27.6	23.6	21.6	21.9	22.2	7.2	7.4	7.7	7.5	7.6	0.5	4.7	2.3	1.2	2.8
Beresford, SD	17.1	18.5	19.2	17.2	18.5	7.5	8.2	8.0	7.6	7.8	2.9	8.0	13.7	3.3	4.4
Lennox, SD	18.0	18.5	20.4	18.2	19.9	7.4	7.7	8.1	7.8	8.1	0.5	2.0	7.0	3.9	5.6
Winter															
Jonestown, MS	12.5	4.0	5.5	3.0	6.5	7.9	8.1	8.1	8.5	8.2	1.9	7.1	7.5	7.3	7.6
Shelby, MS	8.3	2.3	3.3	2.2	3.0	8.0	8.3	8.8	9.2	9.4	1.5	8.8	7.0	8.3	10.2
El Paso, TX	21.2	11.4	-	11.1	-	7.3	7.4	-	8.1	-	0.0	2.7	-	2.6	-
Kermit, TX	17.4	9.8	10.5	8.1	9.1	7.1	7.2	7.1	7.5	7.4	0.3	0.3	0.3	4.3	6.3
Beresford, SD	8.8	1.2	1.9	1.6	2.3	7.7	7.2	7.3	7.4	7.4	4.3	0.1	0.1	0.1	0.1
Lennox, SD	9.4	1.5	2.0	1.2	1.7	7.8	7.5	7.5	7.4	7.5	2.5	0.1	0.1	0.1	0.1
Spring															
Jonestown, MS	20.7	20.7	21.3	20.7	21.3	7.1	8.2	7.8	8.2	7.6	1.4	10.1	14.3	10.0	13.3
Shelby, MS	21.3	22.0	23.0	21.0	23.3	6.5	8.0	8.6	8.0	9.2	2.3	12.1	12.0	12.2	9.8
El Paso, TX	25.5	18.8	-	18.3	-	7.3	7.7	-	7.7	-	0.1	2.2	-	2.2	-
Kermit, TX	21.1	16.9	18.1	16.9	17.8	7.2	7.2	7.2	8.2	8.2	0.3	3.6	2.6	11.7	11.3
Beresford, SD	10.9	15.1	17.1	14.2	16.7	7.7	8.1	8.1	8.1	8.1	3.3	9.6	11.6	6.6	7.6
Lennox, SD	12.0	15.0	16.5	14.6	17.0	7.8	7.8	7.7	8.1	8.1	1.5	0.2	0.2	8.1	9.2

(2) Data on influent virus levels were compared to effluent levels taken approximately 50 days later. This lag approximates the typical average residence times for these systems (TABLE 5), so that influent and effluent represent, at least theoretically, the same mass of water. Because the second method of data analysis yields far fewer comparisons of virus removal and because no statistically significant differences in virus reduction could be discerned, same day comparisons have been more extensively analyzed and will be discussed in detail.

Mean enteric virus levels and percent virus reduction for each site in each season using same day comparison are shown in TABLE 9. All virus determinations used here were plaque counts at 5-days incubation on BGM cell monolayers. Significant virus levels were present in all influent wastewaters throughout the year. However these levels were substantially higher in summer with a mean of 348 PFU/L and were, on the average, lowest in spring with a mean of 55 PFU/L. Levels of virus leaving the systems (pond 2 effluent) were generally similar regardless of season or region. However, effluent levels were consistently higher at the Kermit site throughout the year and at the South Dakota sites in winter. Usually the bulk of the virus removal occurred within the first pond of each system. This effect cannot be explained on the basis of excess retention time in the first pond. The data of TABLE 5 reveal that retention time in the first pond ranged from approximately 40 to 70 percent of total retention time for the first two ponds. Overall virus removal was good; mean percentage removal ranged from 99.28 to 99.96 in summer, from 93.87 to 99.07 in winter and from 89.29 to 99.64 in spring.

In TABLE 10, all 5-day virus removal assay data for all seasons are pooled by site. Mean overall percent removal varied from 93.30 at Beresford to 99.56 at Jonestown. Again it was seen that the greatest contribution to virus reduction occurred in the first lagoons. Except for the El Paso system, no more than 70 percent of virus carried over from the first ponds was removed in the second ponds. Total virus removal appeared to be greater at the southeastern sites, though this effect could not be confirmed by statistical analysis.

The analysis of variance (ANOVA) was run on the individual same-day percentage BGM virus reductions, transformed by arcsin. Effects with p-values less than 0.05 were judged statistically significant. A seasonal difference was detected for pond 1 ($p = 0.048$) and for the total pond system ($p = 0.018$). No other effects were statistically detected (TABLE 11). No interaction of season by region was detected, which indicated that the seasonal differences do not depend on region.

No correlations were detected between percentage virus removal and average physical/chemical parameters either within each season or across seasons. Virus removal was not highly correlated with fecal coliform removal. Fecal coliforms averaged consistently well over two logs removal (99.0%) or greater for all three seasons. Correlations could have been present which were not statistically detected due to the small sample sizes in the study.

TABLE 9. NONAERATED SYSTEMS: ENTERIC VIRUS REMOVAL AS DETERMINED ON BGM CULTURES*

Site and Season	Region	Infl. PFU/L Geometric Mean	Effluent PFU/L Geometric Mean		Percent Total Virus Removal		
			Pond I	Pond II	Pond I	Pond II	Total
Summer							
Jonestown, MS	S.E.	514	4.8	0.2	99.07	95.83	99.96
Shelby, MS	S.E.	791	12.7	0.8	98.40	95.70	99.90
El Paso, TX	S.W.	348	4.6	0.6	98.70	86.96	99.83
Kermit, TX	S.W.	180	7.5	1.3	95.84	82.67	99.28
Beresford, SD	N. Central	94	0.6	0.5	99.36	16.7	99.47
Lennox, SD	N. Central	162	0.5	0.3	99.69	40.0	99.82
Mean		348	5.1	0.6	98.52	69.32	99.71
Winter							
Jonestown, MS	S.E.	32	1.1	0.3	96.6	72.73	99.07
Shelby, MS	S.E.	52	2.6	0.7	95.0	73.08	98.66
El Paso, TX	S.W.	87	2.6	1.0	97.0	61.54	98.85
Kermit, TX	S.W.	148	33.6	9.1	77.4	72.92	93.87
Beresford, SD	N. Central	44	15.2	2.2	65.8	85.53	95.06
Lennox, SD	N. Central	216	11.3	10.5	94.8	7.08	95.13
Mean		97	11.1	4.0	88.20	62.82	96.77
Spring							
Jonestown, MS	S.E.	83	0.5	0.3	99.41	40.0	99.64
Shelby, MS	S.E.	53	0.3	0.2	99.44	33.3	99.63
El Paso, TX	S.W.	74	14.3	1.1	80.6	92.31	98.51
Kermit, TX	S.W.	52	11.7	5.6	77.4	52.16	89.29
Beresford, SD	N. Central	50	2.0	0.4	96.0	20.0	99.21
Lennox, SD	N. Central	17	1.2	0.3	92.8	25.0	98.19
Mean		55	5.0	1.3	90.92	62.43	97.40

* Virus enumerated at 5-days incubation on cell monolayers.

TABLE 10. NONAERATED SYSTEMS: COMBINED ENTERIC VIRUS REMOVAL FOR ALL SEASONS*

Site	Mean % Virus Removal		
	1st Lagoon	2nd Lagoon	Total
Jonestown, MS	98.36	69.50	99.56
Shelby, MS	97.61	66.70	99.41
El Paso, TX	92.10	80.27	99.07
Kermit, TX	83.55	69.20	94.13
Beresford, SD	88.00	47.60	93.30
Lennox, SD	95.77	41.33	97.70

* Virus enumerated at 5-days incubation on BGM monolayers.

TABLE 11. NONAERATED SYSTEMS: P-VALUES FROM ANOVA PROCEDURE AFTER ARCSIN TRANSFORMATION, BGM CELL LINE

Effect	Across Pond I	Across Both Ponds
Season	0.048*	0.018*
Region	0.17	0.33
Site (Region)	0.33	0.15
Season x Region	0.21	0.14

* $p < 0.05$ Judged statistically significant.

In TABLE 12 mean overall virus removals calculated from 50-day residence time sampling analyses are compared to those from same-day sampling analyses. Statistical analysis revealed no significant difference in percent removal by these two techniques of comparison.

TABLE 12. NONAERATED SYSTEMS: COMPARISON OF PERCENT VIRUS REMOVAL FOR SAME DAY VS. 50-DAY LAG ANALYSES*

Season	Mean	Std. Deviation	95% Confidence Limits
Same Day			
Summer	99.7	0.28	99.2-99.9
Winter	94.5	6.69	88.5-99.5
Spring	97.4	4.03	94.3-99.9
Overall	97.2	4.77	96.6-99.5
50-Day Lag			
Summer	98.3	2.65	95.9-99.9
Winter	95.7	4.21	91.3-99.6
Spring	93.4	5.32	85.9-99.1
Overall	95.9	4.34	94.9-98.8

* Virus enumerated at 5-days incubation on BGM cell monolayers.

Results of virus enumeration by region and season on BGM cells are compared to those obtained on the other cell lines in TABLE 13. The ANOVA procedure results are listed in TABLE 14. Virus recoveries from influent (raw wastewater) and from second pond effluent are shown, as are overall percentages of virus removal. There was no consistent difference among cell lines in either virus recovery or indicated virus removal. Furthermore, striking parallels between results from different cell lines can be seen, e.g., in the contrast between the southeast and southwest regions in the spring season. Nevertheless, when individual seasons or regions are considered, some minor divergence appears between the HeLa cell plaque counts and those from the two other cell lines. The seasonal fluctuation of influent virus levels was not apparent in the HeLa cell results (TABLE 14). Also, virus recoveries from winter time effluent samples on HeLa cells tended to be lower than indicated by BGM or RD cell plaque counts. Similarly, there appears in the HeLa cell results less seasonal difference in percent overall virus reduction.

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TABLE 13. NONAERATED SYSTEMS: COMPARISON OF INFLUENT AND EFFLUENT ENTERIC VIRUS LEVELS (GEOMETRIC MEANS) AND OVERALL PERCENT REMOVAL AS DETERMINED ON VARIOUS CELL LINES

Region	BGM			HeLa			RD		
	PFU/L		%	PFU/L		%	PFU/L		%
	Infl.	Effl.	Removal	Infl.	Effl.	Removal	Infl.	Effl.	Removal
Summer									
S.E.	652	0.50	99.94	70.5	0.27	99.62	160	0.25	99.84
S.W.	264	0.95	99.56	28.5	0.56	98.04	271	0.43	99.84
N. Central	128	0.40	99.63	34.0	0.34	99.00	133	0.24	99.82
Winter									
S.E.	42.3	0.50	98.82	30.0	0.21	99.30	37.9	0.33	99.13
S.W.	118	5.1	96.34	48.0	0.74	98.46	63.9	4.66	92.71
N. Central	130	4.9	95.10	83.8	1.04	98.76	60.1	3.07	94.89
Spring									
S.E.	68.4	0.25	99.59	106.0	0.27	99.75	207.0	0.25	99.89
S.W.	62.8	3.4	93.89	40.8	1.76	95.69	35.8	1.18	96.70
N. Central	33.4	0.35	98.74	43.9	0.79	98.20	30.4	0.22	99.28

TABLE 14. NONAERATED SYSTEMS: P-VALUES FROM ANOVA PROCEDURE AFTER ARCSIN TRANSFORMATION, HeLa AND RD CELL LINES

Effect	Across Pond 1	Across Both Ponds
	HeLa Cells	
Season	0.96	0.58
Region	0.09	0.36
Site (region)	0.92	0.26
Season x region	0.72	0.72
	RD Cells	
Season	0.03*	0.046*
Region	0.36	0.23
Site (region)	0.15	0.40
Season x region	0.0007*	0.25

* $p < 0.05$ Judged statisticallly significant.

Thus, indicated virus removal for the summer season was somewhat low and winter time virus removal was relatively better compared to results from BGM and RD cells. Mean percent virus reduction from BGM data ranged from 93.89 to 99.94; from HeLa cells, 95.69 to 99.75; and from RD cells, 92.71 to 99.89.

Although the standard incubation time for all virus-infected cell cultures used in this study was 5 days, plaque assays were held for longer periods, ranging up to 9 days when the integrity of the monolayers persisted. This afforded the advantage of assuring that maximal attainable plaque counts were reached; however, productive incubation time was quite variable and usually was limited by degeneration of the cell monolayer often soon after 5-days incubation. In all cases where prolonged incubation plaque counts were obtained for both influent and corresponding pond 2 effluent, the indicated percent virus reduction was calculated. Apparent reduction of enteric virus by treatment in the lagoons was affected very little by substituting data from prolonged plaque assay incubation periods. Of the acceptable comparisons 35 percent show no significant change, 13 percent show a slight decrease, while 52 percent or about half the prolonged incubation samples show slight (<0.5 Log_{10}) improvement in indicated percent virus removal.

SYSTEMS USING MECHANICAL AERATION

Physical, Chemical and Bacteriological Water Quality Parameters

Observation of various water quality parameters measured simultaneously with virus samples at Muskegon, MI, and Castle Rock, CO, are given in TABLE 15. Data from all three sample points in each system are included, as

TABLE 15. PARTIALLY AERATED SYSTEMS: WASTEWATER PHYSICAL/CHEMICAL CHARACTERISTICS AND FECAL COLIFORMS

Site/ Sampling Date	TOC mg/L Infl.	TOC mg/L Aer.*	TOC mg/L Effl.	TOC % Removal	SS mg/L Infl.	SS mg/L Aer.	SS mg/L Effl.	SS % Removal	TDS mg/L Infl.	TDS mg/L Aer.	TDS mg/L Effl.	TDS % Removal	Conductivity umho/cm Infl.	Conductivity umho/cm Aer.	Conductivity umho/cm Effl.	Conductivity % Removal	Fecal Coliforms Log CFU/L Infl.	Fecal Coliforms Log CFU/L Aer.	Fecal Coliforms Log CFU/L Effl.	Fecal Coliforms % Removal
Muskegon, MI																				
30 Apr	183	128	43	77	187	129	14	93	958	747	694	27.6	1,007	913	858	14.8	6.9	6.0	3.3	99.99
25 Jul	131	125	58	56	140	86	5	96	1,430	926	782	45.3	1,490	1,114	1,097	26.4	9.3	8.0	4.4	99.99
1 Oct	77	48	30	61	214	110	8	96	1,684	1,004	740	56.1	1,210	1,160	945	21.9	9.5	6.8	4.6	99.99
Mean	130	100	43	65	181	108	9.0	95	1,357	892	739	43.0	1,236	1,062	967	21.0	8.6	6.9	4.1	99.99
Castle Rock, CO																				
2 May	145	121	51	65	250	220	110	56	490	580	480	-2.0	730	810	760	-4.1	5.2	3.9	3.4	98.2
18 Jul	101	88	56	45	164	164	88	46	370	500	570	-54.1	635	821	843	-32.8	5.2	3.3	4.0	93.9
9 Oct	73	48	47	36	170	100	28	84	370	300	260	29.7	620	580	400	35.5	5.5	5.3	3.7	98.0
Mean	106	86	51	49	195	161	75	62	410	460	437	-8.8	662	737	668	-0.5	5.3	4.2	3.7	97.6

* Water leaving partially aerated portion of system.

are overall percentages of removal. Greatest removal was for fecal coliforms, though that at Castle Rock was somewhat less (93.9 to 98.2 percent) than that at either Muskegon (99.99 percent) or the nonaerated sites (97.5 to >99.99 percent, TABLE 7). Reductions in the other nonvirological parameters were quite comparable to the ranges observed during the summer season at the nonaerated sites.

Consistent reduction in TDS and conductivity was observed at Muskegon but not in the Castle Rock system. Suspended solids removal was also somewhat better at Muskegon (93 to 96 percent as compared to 46 to 84 percent) and consistently met the 30 mg/L standard. Effluent suspended solids at Castle Rock showed the higher levels common in pond systems. For TOC and suspended solids greatest removal occurred in the second lagoons in both systems. At Muskegon, this was true of fecal coliforms as well.

Data on water temperature, pH and DO are given in TABLE 16. Temperatures, including those of the influent water, were higher at Muskegon than at Castle Rock. Wastewater pH was consistently higher at Castle Rock than at Muskegon. Mean values did not, however, exceed 8.8. pH values increased as the wastewater coursed through the systems. Afternoon pH values in system effluents averaged 7.6 at Muskegon and 8.4 at Castle Rock. In the Muskegon system, DO was approximately 0 in the influent and was still very low in wastewater leaving the partially aerated ponds. At both sites this parameter tended to increase from the aerated zone to the outlet. A simultaneous increase in pH and DO from morning to afternoon was strongest in the downstream ponds at the July sampling time. This was more pronounced at Castle Rock than at the Muskegon system.

Enteric Virus Levels and Virus Reduction

On each sampling day at the sites using mechanical aeration, two replicate virus samples from each sample point were concentrated. Virus enumerations from BGM and the geometric means of replicate determinations are shown in TABLE 17. Site means for each sample point and percent virus reduction across each system are also shown. Virus levels in the influent wastewater were quite low in comparison to summer levels at other sites (TABLE 9). At Muskegon, apparent virus levels in the influent were lower than those in water leaving the partially aerated cells. Here the influent receives significant industrial wastes which might include substances toxic to viruses or to the cell cultures used in their enumeration. Nevertheless mean virus levels in water leaving these systems were well within the range observed at the facultative lagoon sites (TABLE 9). These levels were also quite close for the two systems, 0.66 PFU/L at Muskegon and 0.57 PFU/L at Castle Rock.

Apparent overall percent virus removal was less than the summer season values for the nonaerated systems (TABLE 9). This may be due in part to the very low apparent virus levels in the influent to these systems. Also in contrast to observations on the nonaerated systems, virus removal/destruction did not occur preponderantly within the first lagoon. This may be caused by the very short residence time in the first lagoon in the aerated systems (TABLE 5).

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TABLE 16. AERATED SYSTEMS: WASTEWATER CHARACTERISTICS DETERMINED TWICE/SAMPLING DAY

Site/ Sampling Date	Water Temp (°C)						pH						DO (mg/L)					
	Infl.		Aerated		Effl.		Infl.		Aerated		Effl.		Infl.		Aerated		Effl.	
	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
Muskegon, MI																		
30 Apr	23.9	23.8	18	19	11.5	11.5	7.0	-*	7.3	7.1	7.7	7.6	0	-	0.4	0.6	4.1	3.9
25 Jul	28.8	29.0	27	27.5	28.0	29	6.5	6.8	7.0	7.2	7.5	7.7	0	0	0.3	0.2	2.1	2.6
1 Oct	27.3	27.0	23.5	24	15.5	16	7.1	7.3	7.2	7.4	7.6	7.6	0	0	0	0	3.6	3.7
Mean	26.7	26.6	22.8	23.5	18.3	18.8	6.87	7.05	7.17	7.23	7.60	7.63	0	0	0.23	0.40	3.27	3.40
Castle Rock, CO																		
2 May	16	-	14	-	14	-	8.2	-	8.2	-	8.7	-	3.2	-	2.4	-	8.6	-
18 Jul	18	18.9	20	20	20	20.6	7.8	7.8	7.9	8.0	7.8	8.8	2.3	2.0	2.3	2.4	4.0	7.9
9 Oct	19.5	20.0	15	16	14	11	7.2	7.6	7.8	7.9	8.0	8.0	2.7	2.6	4.6	4.9	3.4	3.2
Mean	18.1	19.5	16.3	18.0	16.0	15.8	7.75	7.70	7.97	7.95	8.17	8.40	2.73	2.30	3.10	3.65	5.33	5.55

* Not done.

TABLE 17. AERATED SYSTEMS: ENTERIC VIRUS LEVELS (PFU/L) IN REPLICATE (a and b) SAMPLES AND OVERALL VIRUS REDUCTION, BGM CELL LINE

Site/Date	Influent a	Influent b	Influent Geom. Mean	Aerated a	Aerated b	Aerated Geom. Mean	Effluent a	Effluent b	Effluent Geom. Mean	Reduction %	Reduction Log_{10}
Muskegon											
30 Apr	<2.49	2.57	2.57	3.88	1.70	2.57	<0.97	0.59	0.59	77.0	0.64
25 Jul	1.22	2.31	1.68	4.06	5.57	4.76	0.27	2.30	0.73	56.5	0.36
1 Oct	*	*		0.63	1.14	0.85	<0.19	<0.19	<0.19		
Mean										66.8	0.50
Geom. Mean			2.08			2.18			0.66		
Castle Rock											
2 May	*	*		*	*		*	*			
18 Jul	123	41.2	67.6	21.4	16.6	20.6	1.09	1.64	1.34	98.0	1.70
9 Oct	49.2	5.91	17.1	53.6	1.55	14.0	<0.22	<0.27	0.244	98.6	1.84
Mean										98.3	1.77
Geom. Mean			34.0			17.0			0.57		

* Sample extremely toxic to cell monolayer.

Overall reduction achieved, as determined on BGM cells, was 66.8 percent at Muskegon and 98.3 percent at Castle Rock. The corresponding values derived from HeLa cell data are quite similar: 80.0 percent at Muskegon and 96.2 percent at Castle Rock.

COMPARISON OF BENTONITE AND EPA METHODS OF VIRUS CONCENTRATION

Simultaneous enteric virus determinations on composite influent wastewater samples were made using the bentonite and proposed EPA methods. These comparisons were confined to sites lacking mechanical aeration, but were performed in all three regions of the U.S. where such sites were studied. BGM and HeLa cell lines were used for enumeration of the recovered virus.

The results are shown in TABLE 18, both in PFU/L and as ratios of recovery by the bentonite method compared to recovery by the EPA method. In most comparisons, the bentonite method yielded the higher recovery value. A statistically significant difference in recovery efficiency cannot, however, be shown.

TABLE 18. COMPARISON OF VIRUS RECOVERIES* USING THE BENTONITE AND PROPOSED EPA ($AlCl_3$/BALSTON FILTER) METHODS

Site	Bentonite Method		EPA Method		Bentonite/EPA	
	BGM	HeLa	BGM	HeLa	BGM	HeLa
Jonestown		33.1		5.96		5.55
Shelby	206.0	53.6	77.2	130.0	2.67	0.41
Kermit	113.0	51.9 12.6	396.0	265.0	0.29	0.10
Beresford	19.3 16.2	19.3	2.27	3.03	7.80	6.37
Lennox	187.0	112.0	34.7	13.3	5.40	8.43
Geom. Mean					2.39	1.65

* PFU/L of virus.

During previous studies (to be published) of virus concentration with the bentonite absorption/filtration method, the efficiency of seeded poliovirus recovery was examined. No significant trends or differences in virus recovery were observed between raw and biologically treated secondary wastewater effluent. The efficiency of virus recovery was approximately 40 percent.

QUALITY ASSURANCE SAMPLES; PLAQUE VERIFICATION

For some influent wastewater samples, an aliquot was subjected to virus concentration at the USAMBRDL Laboratory. This served as a quality assurance assessment of the efficiency of the concentration procedure practiced in the field. Mean values by region and season are shown in Table 19. Comparisons were made in all three seasons and used BGM and HeLa cell lines. Ratios of virus recovery following field concentration to that following laboratory concentration ranged, from 0.22 to 1.08. Geometric mean values for the two cell lines did not differ significantly. A concentration efficiency of approximately 50 percent for the large volume field concentration procedure was indicated.

A total of 254 plaques were examined to verify their viral origin by passage of the plaque isolate material on the cell lines to from which they were picked. All major distinguishable plaque morphologies were proportionately represented. Overall percent verification was 92.5. No statistically significant differences in verification were noted according to the geographical site of origin, the sampling point within the pond system, or the cell line from which the original plaque was recovered.

TABLE 19. COMPARISON OF RESULTS OF VIRUS CONCENTRATION OF INFLUENT COMPOSITE QUALITY ASSURANCE SAMPLES IN THE FIELD AND AT THE USAMBRDL LABORATORY (PFU/L)

Season	Region	Concentration in Field		Concentration in Lab		Field/Lab	
		BGM	HeLa	BGM	HeLa	BGM	HeLa
Summer	Southwest	113	26	108	38	1.05	0.67
	North Central	41	23	186	77	0.22	0.30
Winter	Southeast	37	26	95	81	0.39	0.32
	North Central	79	27	107	25	0.74	1.08
Spring	Southwest	216		822		0.26	
	North Central	33		30		1.07	
Geom. Mean						0.514	0.513

SECTION 6

DISCUSSION

This study represents one of the first quantitative evaluations of enteric virus treatability on operational, full-scale wastewater treatment lagoon systems in the United States in which multiple sites, regions, and seasons were examined. A number of previous studies of enteric virus removal in treatment lagoons were qualitative (12,15,16,19), or performed on a bench or pilot scale or in single lagoon systems (10,13,20) and often were directed at the determination of factors contributing to virus removal (11,14,17,18). In general, these studies indicated that under typical operating conditions enteric virus levels were reduced after residence in the lagoons. The principal thrust of this study was to determine if the virus removal characteristics observed by others were uniform and reproducible regardless of site, region, or season in typically designed and operated wastewater treatment lagoon systems. It was not the goal of this project to identify specific factors contributing to virus removal within the lagoon systems.

The study consisted of two parts: a larger study of paired nonaerated pond systems located in three geographic regions of the U.S. and a smaller range-finding study using two systems in which the first one or two ponds in series received partial mechanical aeration. The principal objective of the latter study was to observe any difference in overall virus reduction associated with the use of aeration.

VIRUS REMOVAL IN FACULTATIVE LAGOONS

Virus removal was examined using influent-effluent data pairs from concurrent samples (same day) and data pairs representing a 50-day typical theoretical lagoon system residence period. The two modes of comparison did not reveal any statistically significant differences in estimated virus removal. Therefore, concurrent sample data were used for characterizing virus removal, with the added benefit of providing nearly twice as many comparative data points. An actual evaluation of residence time was not possible without tracer studies, because of short circuiting, effects of wind (wave) action, and variation in hydraulic loading rates.

An important result of this study was the comparative effectiveness of the first pond in each facultative system to remove virus from the wastewater. In general, well over half the total virus removal capability of the first two lagoons in series resided in the first pond year-round. Similar observations were made for TOC, total suspended solids and fecal coliforms. Evidence from other studies on virus removal points to extensive virus association with particulates and subsequent settling as primary removal

factors in the first ponds. Other effects such as high algal and bacterial populations and their metabolic activities cannot be excluded as possible mechanisms active in removal. Temperature, DO, and pH differences between the first and second ponds in these systems were usually very minor and are not considered important factors in the explanation of the differences observed. No significant differences were observed in holding time between first and second ponds to account for differences in virus treatability.

A primary question to be answered in this study was whether or not facultative lagoons could provide good (approximately 95 percent) enteric virus removal regardless of site, region, or season. The average virus removal capabilities demonstrated in the six facultative lagoon systems for the combined seasons ranged from 93.3 to 99.5 percent and during the summer all systems provided greater than 99 percent removal. The virus removals were comparable to those often described for activated sludge and trickling filtration secondary wastewater treatment methods (5). This is significant from a budgetary viewpoint if virus treatability is considered important since lagoon systems generally involve lower design and operating costs and are also easier to operate.

An analysis of variance (ANOVA) did not reveal any statistically significant difference in virus removal with regard to site or region. However, a seasonal impact was observed ($P<0.05$) in which wintertime virus removals within the first ponds and the total lagoon systems were less than in spring and summer.

The virus removal capabilities of the facultative lagoon systems and the effects of temperature noted in this study are similar to those observed in quantitative studies performed by others. Rao (20) observed 88 to 99 percent indigenous virus removal efficiency from raw wastewater in 4 to 5 ft (1.2 to 1.5 m) deep ponds. These removals generally were observed within pilot- and full-scale single pond systems with retention periods of 2.7 to 17.2 days. Water temperatures in the study even in winter were generally between 20° and 35°C; thus, wintertime comparisons to our study were not possible. Sagik et al. (10), using primary and secondary treated wastewater in shallow (<1.1 m deep) pilot-scale single pond systems evaluated seeded enterovirus removal on a seasonal basis. Greater than 99.9 percent virus inactivation occurred within 10 days in secondary effluent when water temperatures exceeded 20°C. At 10°C only 50 percent inactivation was noted in the same period. Virus inactivation in primary wastewater ponds was comparable to secondary ponds during winter but was less during summer. Kott et al. (30) observed that virus within secondary effluent contained in static ponds was reduced by over 95 percent in 47 days when water temperature was around 8°C. During summer, virus levels were reduced at least 95 percent within 11 days when water temperatures were 18 to 22°C. Results of the present study and these other studies identify temperature as a very important consideration in virus removal. Holding (residence) periods to achieve a 95 percent removal might require adjustment with season. Further study at full-scale lagoon sites would permit assessment of the effects of water temperature and associated holding period.

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While seasonal effects on the percent virus removal were noted in this study, it was also observed that the average virus levels found in the wastewater exiting the lagoon systems were uniformly low throughout the year (<2 PFU/L). In summer, influent virus levels were greater, but this was countered by the higher efficiency of virus treatability during that period. During winter, reduced virus treatment efficiency was tempered by reduced virus levels in the influent wastewaters. The low levels of virus detected in the lagoon effluents were noted with all three cell culture assay systems used (BGM, HeLa, and RD). It is probable that the actual virus levels exiting the lagoon systems were at least twice those detected because previous studies in various wastewaters yielded approximately a 40 percent recovery rate using the bentonite method. Additionally, it is possible that very slow plaque-forming viruses (>7 day incubation) and those not infectious for the cell lines used escaped detection.

An ancillary objective of this study was to measure the reduction of certain wastewater quality parameters through the lagoon systems to determine if any were correlated with virus removal. Treatability of the selected wastewater parameters was reasonably uniform from site to site and was consistent with the data obtained from the literature as provided in an excellent review by Sagik et al. (10). This was expected since the systems selected were chosen on the basis of their compliance with state and USEPA criteria for operational characteristics. TOC and total suspended solids removal was commonly over 70 percent during summer, but was more variable in other seasons. Fecal coliform removals were generally over 99 percent throughout the study. Water temperature reflected seasonal conditions. The pond pHs tended to be highest in summer and generally ranged from around 7 to 9. Dissolved oxygen was lowest during winter. Usually the temperature, pH, and DO did not change much from the first to second pond. Elevated pond pH and DO during the sampling days are probably attributable to uptake of CO_2 during algal photosynthesis, as high algal populations in the ponds were obvious.

An analysis of variance was performed on the physical/chemical wastewater characteristics in the lagoon systems but none was correlated significantly with virus. Perhaps a correlation could have been made if further sampling could have been conducted. While the present study provides a general verification of the capabilities of facultative wastewater treatment lagoons to remove enteric viruses before wastewater discharge under typical conditions, the actual mechanisms for removal are not thoroughly understood. Additionally, the impact of adverse conditions in lagoon systems, such as excess hydraulic loading, extended freezing, seasonal turnover and thermal stratification, industrial waste input and toxicity/poisoning of the lagoons has not been evaluated.

VIRUS REMOVAL IN LAGOONS WITH PARTIAL AERATION

The results from the limited study on systems receiving partial aeration indicate no difference in the enteric virus content of effluent between these and the nonaerated systems. The data indicate that, in contrast to the nonaerated systems, virus removal did not occur preponderantly in the first pond of the system. This is probably attributable to the very short residence

times for water in these ponds. Virus reduction within the larger nonaerated portion was comparable to that at the paired facultative sites.

Very low enteric virus levels were observed in the influent wastewater at the sites using aeration. This was especially true at Muskegon, MI. This may have resulted from the presence of toxic materials in these wastewaters. In any case, estimates of percent virus reduction within these systems should be made with the depressed nature of these influent values in mind.

VIRUS HAZARD POTENTIAL AND PROCESS OPTIMIZATION

The presence of low PFU/L levels of enteric virus in lagoon effluents would probably represent a low-level health hazard if the water were released undisinfected to a receiving stream or applied to land. Disinfection to provide a free available chlorine residual of 2 to 5 mg/L would be expected to reduce virus levels by another one to two orders of magnitude, thus essentially eliminating the virus hazard except for direct body contact or consumption. Aerosol hazards from land application of the disinfected effluent by spray irrigation may also be reduced because of additional inactivation or dilution of virus during dispersion in the aerosol.

In order to optimize the virus treatability of lagoon systems, a better understanding of factors contributing to such treatability is necessary. While high temperature (10,12,13), pH >8 (10,18), high suspended solids concentration (11,19), biological antagonism (11), shallow pond depth (10), and wastewater pretreatment (10) all contribute to virus inactivation to various degrees, their impact individually is difficult to assess. It is likely that all of these factors work simultaneously in an additive manner toward virus removal. A better understanding of the roles and interactions of these factors could lead to optimization of virus treatability through modified design and operation of lagoon systems and may help identify additives that would enhance the actions of the above factors.

The role of virus adsorption on wastewater particulates or on algae and other microbiological organisms is probably very important in virus removal from the wastewater, and the need for further studies should be emphasized (10,11,20). These studies should be performed to establish the kinetics of adsorption, the rate of sedimentation, the effects of thermal turnover and excessive hydraulic loading, the rate of inactivation or irreversible solids association, and the potential for enhanced virus association with settleable solids through cation or clay addition, lower pH, or use of coagulant aids.

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REFERENCES

1. Clarke, N. A., G. Berg, P. W. Kabler, and S. L. Chang. Human Enteric Viruses in Water: Source, Survival and Removability. Adv. Water Poll. Res., 2:523-541, 1964.

2. Shuval, H. I. Detection and Control of Enteroviruses in the Water Environment. In: Developments in Water Quality Research. H. I. Shuval, ed., Ann Arbor, MI, Ann Arbor - Humphrey Science Pub., 1970. pp. 47-71.

3. Buras, N. Recovery of Viruses from Wastewater and Effluent by the Direct Inoculation Method. Water Research, 8:19-22, 1974.

4. Sorber, C. A., S. A. Schaub, and H. T. Bausum. An Assessment of a Potential Virus Hazard Associated with Spray Irrigation of Domestic Wastewaters. In: Virus Survival in Water and Wastewater Systems, Water Resources Symposium No. 1, J. F. Malina and B. P. Sagik, eds. Center for Research in Water Resources, University of Texas, Austin, TX, 1974. pp. 241-252.

5. Feachem, R., H. Garelick, and J. Slade. Enteroviruses in the Environment. Tropical Diseases Bull, 78(3):185-230, 1981.

6. Bausum, H. T., S. A. Schaub, and K. F. Kenyon. Viral and Bacterial Aerosols at a Wastewater Spray Irrigation Site. Technical Report 7804 (AD A034436), US Army Medical Bioengineering Research and Development Laboratory. Ft. Detrick, Frederick, MD, 1978. 37 pp.

7. Johnson, D. E., D. E. Camann, C. A. Sorber, B. P. Sagik, and J. P. Glennon. Aerosol Monitoring for Microbial Organisms Near a Spray Irrigation Site. In: Proc. Conf. on Risk Assessment and Health Effects of Land Application of Municipal Wastewater. B. P. Sagik and C. A. Sorber, eds. Center for Applied Research and Technology, University of Texas at San Antonio, TX, 1978. pp. 231-239.

8. Wellings, F. M., A. L. Lewis, C. W. Mountain, and L. V. Pierce. Demonstration of Virus in Groundwater after Effluent Discharge onto Soil. Appl. Environ. Microbiol., 29(6):751-757, 1975.

9. Schaub, S. A., and C. A. Sorber. Virus and Bacteria Removal from Wastewater by Rapid Infiltration Through Soil. Appl. Environ. Microbiol. 33(3):609-619, 1975.

10. Sagik, B. P., S. W. Funderburg, B. E. Moore, R. C. Tripathi, and C. A. Sorber. The Survival of Human Enteric Viruses in Holding Ponds. Report No. 78-1, Center for Applied Research and Technology, University of Texas, San Antonio, 1978. 193 pp.

11. Sobsey, M. D., and R. C. Cooper. Enteric Virus Survival in Algal-Bacterial Wastewater Treatment Systems - I. Water Research 7:669-685, 1973.

12. Slanetz, L. W., C. H. Bartley, T. G. Metcalf, and R. Nesman. Survival of Enteric Bacteria and Viruses in Municipal Sewage Lagoons. In: Proceedings of the Second International Symposium for Waste Treatment Lagoons, R. E. McKinney, ed. Missouri Basin Engineering Health Council, Kansas City, MO, 1970. pp. 132-141.

13. Kott, Y., H. Ben-Ari, and N. Betzer. Lagooned Secondary Effluents as Water Source for Extended Agricultural Purposes. Water Research, 12:1101-1106, 1978.

14. Malina, J. F. Application of Oxygen to Treat Waste from Military Field Installations. Report CRWR - 99. Center for Research in Water Resources, Civil Engineering Department, University of Texas, Austin, TX, 1973. 77 pp.

15. Englande, B., R. E. Leach, B. Adams, and R. Schiosaki. Virological Assessment of Sewage Treatment at Santee, CA. In: Transmission of Viruses by the Water Route, G. Berg, ed. Interscience Pub., New York, 1967. pp. 401-417.

16. BoPardikar, M. V. Microbiology of a Waste Stabilization Pond. In: Advances in Water Pollultion Research. Proceedings Fourth International Conference Program, S. A. Jenkins, ed. Pergamon Press, Oxford, 1969. pp. 595-601.

17. Nupen, E. M., B. W. Bateman, and N. C. McKenny. The Reduction of Virus by the Various Unit Processes Used in the Reclamation of Sewage to Potable Waters. In: Virus Survival in Water and Wastewater Systems, Water Resources Symposium, No. 7, J. F. Malina and B. P. Sagik, eds. Center for Research in Water Resources, University of Texas, Austin, TX, 1973. pp. 110-114.

18. Idelovitch, E. Wastewater Reuse by Biological-Chemical Treatment and Groundwater Recharge. J. Water Poll. Control Fed., 50:2723-2740, 1978.

19. Christie, A. E. Virus Reduction in the Oxidation Pond. Water and Poll. Control, 105(5):45-54, 1967.

20. Rao, V. P., S. B. Lakhe, and S. V. Waghmare. Virus Removal in Waste Stabilization Ponds in India. Water Research, 15:773-778, 1981.

21. Katzenelson, E., B. Fattal, and T. Hostovesky. Organic Flocculation: An Efficient Second Step Concentration Method for the Detection of Viruses in Tapwater. Appl. Environ. Microbiol., 32(4):638-639, 1976.

22. American Public Health Association. Standard Methods for the Examination of Water and Wastewater, 14th Ed. American Public Health Association, Washington, DC, 1976.

23. Box, G.E.P., W.G. Hunter, and J.S. Hunter. 1978. Statistics for experimenters. John Wiley & Sons, New York, NY.

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APPENDIX A

FACULTATIVE PONDS DATA

The purpose of this compilation is to provide a fuller presentation of the data obtained from the six facultative pond sites. Data on physical/chemical parameters, fecal coliforms and virus are included. For the aerated systems a comparable level of detail has been provided in the main body of the report (TABLES 15-17).

In the tables that follow, mean levels for each measured parameter are given for each study site in each season. Minimum and maximum values observed within each season are also included, as are percent removal for the first pond and total percent removal.

Tables assigned to the various physical/chemical parameters and fecal coliforms are as follows:

Suspended solids	(TABLE A-1)
Total dissolved solids	(TABLE A-2)
Electrical conductivity	(TABLE A-3)
Total organic carbon	(TABLE A-4)
Fecal coliforms	(TABLE A-5)
Water temperature	(TABLE A-6)
pH	(TABLE A-7)
Dissolved oxygen	(TABLE A-8)

Tables presenting virus data obtained from the various cell lines used are:

Virus data, BGM cells	(TABLE A-9)
Virus data, HeLa cells	(TABLE A-10)
Virus data, RD cells	(TABLE A-11)

TABLE A-1. MEAN LEVELS (mg/L) AND PERCENT REMOVAL OF SUSPENDED SOLIDS, NONAERATED SYSTEMS

Season/Site	Influent			Pond I Effluent			Percent Removal	Pond II Effluent			Overall Percent Removal
		Range			Range				Range		
	Mean	Min	Max	Mean	Min	Max		Mean	Min	Max	
Summer											
Jonestown, MS	165	112	271	116	40	185	30	70	51	98	58
Shelby, MS	143	98	194	101	68	132	29	56	44	73	61
El Paso, TX	168	160	175	81	75	85	52	5	4	7	97
Kermit, TX	149	65	298	136	88	204	9	45	35	55	70
Beresford, SD	102	65	129	57	32	101	45	6	2	12	94
Lennox, SD	81	65	90	52	18	90	35	41	20	71	49
Winter											
Jonestown, MS	84	62	105	70	58	82	16	72	65	78	14
Shelby, MS	136	95	163	112	90	128	18	114	85	133	17
El Paso, TX	132	120	140	126	110	140	5	134	118	157	-1
Kermit, TX	249	131	332	54	40	66	78	73	65	83	71
Beresford, SD	132	117	151	11	9	13	92	17	8	31	87
Lennox, SD	185	150	229	16	14	17	92	16	11	25	91
Spring											
Jonestown, MS	225	119	395	104	68	152	54	70	41	99	69
Shelby, MS	69	33	97	87	67	112	-25	60	31	111	13
El Paso, TX	154	148	165	142	110	173	8	139	97	168	10
Kermit, TX	200	55	365	97	68	120	52	186	126	272	7
Beresford, SD	146	131	153	67	33	133	54	25	7	38	83
Lennox, SD	143	142	145	23	19	27	84	39	17	53	73

TABLE A-2. MEAN LEVELS (mg/L) AND PERCENT REMOVAL OF TOTAL DISSOLVED SOLIDS NONAERATED SYSTEMS

Season/Site	Influent Mean	Influent Range Min	Influent Range Max	Pond I Effluent Mean	Pond I Effluent Range Min	Pond I Effluent Range Max	Percent Removal	Pond II Effluent Mean	Pond II Effluent Range Min	Pond II Effluent Range Max	Overall Percent Removal
Summer											
Jonestown, MS	460	394	558	372	320	410	19	330	286	372	29
Shelby, MS	751	543	840	766	734	803	-2	735	695	790	2
El Paso, TX	845	825	860	758	750	765	10	695	650	725	19
Kermit, TX	666	548	782	676	630	770	-2	691	642	714	-4
Beresford, SD	1,478	1,360	1,678	1,419	1,329	1329	4	1,546	1,399	1,822	-5
Lennox, SD	2,973	2,777	3,214	3,125	2,841	3,636	-5	3,217	2,992	3,473	-8
Winter											
Jonestown, MS	481	405	557	423	326	520	12	374	363	384	22
Shelby, MS	912	736	1,077	751	669	894	18	681	601	738	25
El Paso, TX	723	690	760	725	700	755	0	725	700	755	0
Kermit, TX	557	490	638	603	600	606	-8	628	602	652	-13
Beresford, SD	1,295	1,183	1,371	1,457	1,123	1,640	-13	1,452	1,167	1,613	-12
Lennox, SD	3,043	2,818	3,216	3,141	2,932	3,361	-3	3,093	2,627	3,373	-2
Spring											
Jonestown, MS	574	552	616	368	339	412	36	311	229	390	46
Shelby, MS	799	713	879	573	518	639	28	551	493	640	31
El Paso, TX	750	710	780	747	715	765	0	777	720	810	-4
Kermit, TX	489	466	526	600	596	607	-23	580	562	612	-19
Beresford, SD	1,603	1,372	1,770	1,487	1,141	1,828	7	1,425	1,222	1,690	11
Lennox, SD	3,249	2,843	3,690	3,197	2,950	3,361	2	2,863	2,523	3,280	12

TABLE A-3. MEAN LEVELS (μmho/cm) AND PERCENT REMOVAL OF CONDUCTIVITY, NONAERATED SYSTEMS

Season/Site	Influent			Pond I Effluent			Percent Removal	Pond II Effluent			Overall Percent Removal
		Range			Range				Range		
	Mean	Min	Max	Mean	Min	Max		Mean	Min	Max	
Summer											
Jonestown, MS	595	510	670	408	350	450	31	372	310	420	37
Shelby, MS	948	580	1,180	850	780	900	10	808	700	870	15
El Paso, TX	750	750	750	703	700	710	6	697	690	700	7
Kermit, TX	1,039	856	1,221	1,047	948	1,202	-1	1,078	1,002	1,115	-4
Beresford, SD	2,120	1,830	2,850	1,905	1,570	2,400	10	1,858	1,680	2,100	12
Lennox, SD	3,775	3,070	4,900	3,883	3,220	5,150	-3	3,730	3,120	4,850	1
Winter											
Jonestown, MS	570	535	605	500	475	525	12	450	400	500	21
Shelby, MS	1,037	945	1,115	885	735	1,130	15	812	755	841	22
El Paso, TX	1,083	1,000	1,150	1,083	1,050	1,100	0	1,092	1,050	1,125	0
Kermit, TX	870	766	996	941	935	946	-8	981	940	1,019	-13
Beresford, SD	2,030	1,890	2,300	2,207	1,670	2,650	-9	2,137	1,600	2,700	-5
Lennox, SD	3,880	3,500	4,290	4,197	3,780	4,410	-8	4,057	3,720	4,300	-5
Spring											
Jonestown, MS	642	620	655	360	360	360	44	357	350	360	44
Shelby, MS	995	840	1,155	692	680	715	30	628	590	695	37
El Paso, TX	1,177	1,160	1,210	1,175	1,160	1,200	0	797	140	1,200	32
Kermit, TX	763	728	822	936	931	947	-22	904	878	956	-18
Beresford, SD	2,357	2,040	2,550	2,150	1,850	2,600	8	2,107	1,920	2,450	11
Lennox, SD	4,320	3,700	5,400	4,307	4,000	4,720	0	3,970	3,660	4,400	8

TABLE A-4. MEAN LEVELS (mg/L) AND PERCENT REMOVAL OF TOTAL ORGANIC CARBON, NONAERATED SYSTEMS

Season/Site	Influent			Pond I Effluent			Percent Removal	Pond II Effluent			Overall Percent Removal
		Range			Range				Range		
	Mean	Min	Max	Mean	Min	Max		Mean	Min	Max	
Summer											
Jonestown, MS	84	36	123	49	33	82	42	38	19	73	55
Shelby, MS	150	64	278	92	27	230	38	67	25	179	55
El Paso, TX	110	71	156	88	83	93	20	62	57	71	43
Kermit, TX	100	72	121	65	51	75	35	55	47	72	45
Beresford, SD	77	71	83	46	20	63	40	26	20	31	66
Lennox, SD	76	57	90	47	32	65	38	36	32	44	53
Winter											
Jonestown, MS	77	41	122	41	31	55	47	31	25	40	60
Shelby, MS	86	59	130	31	23	40	64	31	20	50	63
El Paso, TX	79	65	90	64	40	83	20	56	40	67	30
Kermit, TX	98	63	125	56	48	65	42	44	40	48	55
Beresford, SD	58	45	77	51	40	61	11	44	23	75	24
Lennox, SD	104	78	138	56	40	66	46	47	33	61	55
Spring											
Jonestown, MS	135	89	180	57	28	86	58	22	21	22	84
Shelby, MS	112	75	140	57	25	75	49	28	13	42	75
El Paso, TX	72	51	109	69	56	86	3	57	45	79	20
Kermit, TX	107	99	120	61	55	68	43	48	33	60	55
Beresford, SD	64	56	77	43	36	55	33	28	20	34	56
Lennox, SD	94	85	104	43	39	46	54	42	18	54	55

TABLE A-5. MEAN LEVELS (LOG_{10}) AND PERCENT REMOVALS OF FECAL COLIFORMS, NONAERATED SYSTEMS

Season/Site	Influent			Pond I Effluent			Percent Removal	Pond II Effluent			Overall Percent Removal
		Range			Range				Range		
	Mean	Min	Max	Mean	Min	Max		Mean	Min	Max	
Summer											
Jonestown, MS	7.62	6.90	8.26	4.80	4.15	5.34	99.85	4.35	3.30	5.26	99.95
Shelby, MS	6.95	6.43	7.94	4.47	3.69	5.16	99.67	3.68	3.20	3.92	99.95
El Paso, TX	6.32	5.54	6.87	4.51	4.13	5.23	98.44	4.41	4.08	5.08	98.76
Kermit, TX	6.42	6.23	6.68	4.49	1.30	6.04	98.82	4.39	3.73	4.72	99.07
Beresford, SD	6.57	6.15	7.00	4.93	4.68	5.28	97.71	2.47	1.30	3.11	99.99
Lennox, SD	6.82	6.67	7.04	5.17	4.85	5.40	97.77	2.45	1.30	3.60	99.995
Winter											
Jonestown, MS	6.39	6.34	6.45	4.44	4.34	4.54	98.88	3.35	3.34	3.36	99.91
Shelby, MS	6.63	6.26	7.19	4.47	4.08	4.98	99.39	3.84	3.38	4.23	99.83
El Paso, TX	6.05	6.04	6.08	2.11	1.98	2.24	99.99	0.72	0.48	1.08	99.999
Kermit, TX	6.95	6.51	7.22	6.04	5.95	6.15	87.76	4.89	4.30	5.36	99.14
Beresford, SD	6.48	6.15	6.82	5.31	5.11	5.66	93.26	4.11	3.85	4.32	99.57
Lennox, SD	6.87	6.72	7.15	5.66	5.13	6.11	93.81	5.27	5.00	5.57	97.48
Spring											
Jonestown, MS	7.04	6.98	7.15	4.33	3.15	4.94	99.81	3.46	2.68	3.88	99.97
Shelby, MS	6.82	6.66	6.98	3.88	3.23	5.00	99.89	3.07	2.60	4.00	99.98
El Paso, TX	8.03	6.88	8.93	3.55	2.23	4.22	99.99	2.51	1.23	3.15	99.999
Kermit, TX	6.84	6.65	6.97	5.57	5.00	6.00	94.60	3.53	3.00	4.30	99.95
Beresford, SD	6.37	6.08	6.72	4.72	4.34	4.93	97.74	1.93	1.60	2.41	99.995
Lennox, SD	7.04	6.72	7.26	5.10	4.98	5.32	98.87	2.94	2.43	3.78	99.99

TABLE A-6. MEAN WATER TEMPERATURE (^{o}C) AND TEMPERATURE DIFFERENCES FROM INFLUENT TO LAGOONS IN NONAERATED SYSTEMS

Season/Site	Influent			Pond I Effluent				Pond II Effluent			Overall
		Range			Range				Range		
	Mean	Min	Max	Mean	Min	Max	Δ Temp	Mean	Min	Max	Δ Temp
Summer, Morning											
Jonestown, MS	27	26	28	30	30	30	3	30	29	31	3
Shelby, MS	28	27	31	29	23	33	1	28	21	32	0
El Paso, TX	29	26	31	28	26	31	0	28	26	31	-1
Kermit, TX	28	24	34	24	15	34	-4	22	15	28	-6
Beresford, SD	17	16	18	18	11	25	1	17	10	22	0
Lennox, SD	18	17	19	18	10	24	0	18	10	24	0
Winter, Morning											
Jonestown, MS	13	11	14	4	3	5	-9	3	2	4	-10
Shelby, MS	8	-1	15	2	-1	4	-6	2	0	4	-6
El Paso, TX	21	18	23	11	10	14	-10	11	8	13	-10
Kermit, TX	17	16	18	10	9	11	-8	8	7	9	-9
Beresford, SD	9	7	10	1	0	2	-8	2	1	2	-7
Lennox, SD	9	8	11	2	1	2	-8	1	0	2	-8
Spring, Morning											
Jonestown, MS	21	13	28	21	14	26	0	21	14	26	0
Shelby, MS	21	16	29	22	16	29	1	21	16	26	0
El Paso, TX	26	23	28	19	17	22	-7	18	17	21	-7
Kermit, TX	21	18	24	17	13	21	-4	17	13	21	-4
Beresford, SD	11	8	13	15	7	22	4	14	6	21	3
Lennox, SD	12	10	15	15	7	21	3	15	7	21	3
Summer, Afternoon											
Jonestown, MS	27	26	28	32	31	34	5	32	28	34	4
Shelby, MS	28	28	39	32	27	34	4	31	27	34	3
El Paso, TX	.*	.	.	29	26	31	-	28	26	31	-
Kermit, TX	27	23	29	22	17	25	-5	22	17	25	-5
Beresford, SD	.	.	.	19	12	23	-	19	11	25	-
Lennox, SD	.	.	.	20	12	25	-	20	12	25	-
Winter, Afternoon											
Jonestown, MS	12	12	12	6	5	6	-7	7	6	7	-6
Shelby, MS	16	16	16	3	1	5	-13	3	1	4	-13
El Paso, TX	.	.	.	.	.	.	-	.	.	.	-
Kermit, TX	.	.	.	11	9	12	-	9	8	10	-
Beresford, SD	.	.	.	2	0	3	-	2	2	3	-
Lennox, SD	.	.	.	2	1	3	-	2	1	3	-
Spring, Afternoon											
Jonestown, MS	.	.	.	21	14	30	-	21	15	30	-
Shelby, MS	22	14	30	23	15	35	1	23	16	34	1
El Paso, TX	.	.	.	.	.	.	-	-	-	-	-
Kermit, TX	.	.	.	18	14	22	-	18	13	22	-
Beresford, SD	.	.	.	17	8	24	-	17	7	23	-
Lennox, SD	.	.	.	17	8	23	-	17	8	24	-

* Not done.

TABLE A-7. MEAN pH VALUES AND pH DIFFERENCES FROM INFLUENT TO LAGOONS IN NONAERATED SYSTEMS

Season/Site	Influent			Pond I Effluent				Pond II Effluent			Overall
		Range			Range				Range		
	Mean	Min	Max	Mean	Min	Max	Δ pH	Mean	Min	Max	Δ pH
Summer, Morning											
Jonestown, MS	7.7	7.4	8.0	8.2	8.0	8.6	1.5	7.9	7.6	8.2	0.2
Shelby, MS	7.7	7.1	8.3	9.3	8.3	10.4	1.6	9.4	7.4	10.4	1.8
El Paso, TX	7.2	7.0	7.6	8.0	7.8	8.1	0.8	8.2	8.1	8.3	1.0
Kermit, TX	7.2	7.1	7.3	7.4	7.3	7.7	0.3	7.5	7.3	7.7	0.4
Beresford, SD	7.5	7.5	7.7	8.2	7.7	8.7	0.6	7.6	7.3	8.0	0.0
Lennox, SD	7.4	6.7	7.8	7.7	7.1	8.3	0.4	7.8	7.3	8.5	0.4
Winter, Morning											
Jonestown, MS	7.9	7.6	8.2	8.1	7.7	8.5	0.2	8.5	8.2	8.7	0.6
Shelby, MS	8.0	7.9	8.3	8.3	8.1	8.6	0.3	9.2	8.5	10.4	1.20
El Paso, TX	7.3	7.0	7.6	7.4	7.2	7.6	0.1	8.1	7.6	8.6	0.9
Kermit, TX	7.1	7.1	7.2	7.2	7.1	7.3	0.1	7.5	7.4	7.5	0.4
Beresford, SD	7.7	7.6	7.8	7.2	7.1	7.4	-0.5	7.4	7.2	7.6	-0.4
Lennox, SD	7.9	7.8	7.9	7.5	7.4	7.7	-0.4	7.4	7.4	7.5	-0.4
Spring, Morning											
Jonestown, MS	7.1	5.7	7.8	8.2	7.2	9.6	1.2	8.2	7.2	9.5	1.2
Shelby, MS	6.5	4.7	7.6	8.0	7.5	8.7	1.5	8.0	7.8	8.2	1.5
El Paso, TX	7.3	7.1	7.6	7.7	7.6	7.8	0.4	7.7	7.6	7.8	0.4
Kermit, TX	7.2	7.1	7.5	7.2	7.1	7.3	-0.1	8.2	7.5	8.5	0.9
Beresford, SD	7.7	7.6	7.9	8.1	7.6	8.9	0.4	8.1	7.7	8.5	0.4
Lennox, SD	7.8	7.7	8.0	7.8	7.7	8.0	0.0	8.1	7.7	8.7	0.3
Summer, Afternoon											
Jonestown, MS	7.9	7.2	8.9	9.1	8.7	9.6	1.2	8.4	7.8	9.4	0.5
Shelby, MS	8.2	7.9	8.7	10.0	8.3	10.8	1.9	10.5	10.3	10.9	2.4
El Paso, TX	.*	.	.	8.1	8.0	8.1	-	8.2	8.1	8.3	-
Kermit, TX	7.1	7.0	7.4	7.7	7.3	8.7	0.6	7.6	7.4	7.8	0.5
Beresford, SD	.	.	.	8.0	7.4	8.8	-	7.8	7.6	8.1	-
Lennox, SD	.	.	.	8.0	7.7	8.4	-	8.1	7.7	8.7	-
Winter, Afternoon											
Jonestown, MS	7.7	7.7	7.7	8.1	7.7	8.5	0.4	8.2	8.0	8.3	0.5
Shelby, MS	8.0	7.7	8.3	8.8	8.2	9.1	0.8	9.4	8.6	10.6	1.4
El Paso, TX	.	.	.	.	.	.	-	.	.	.	-
Kermit, TX	7.5	7.4	7.5	7.1	7.1	7.2	-0.3	7.4	7.3	7.5	-0.0
Beresford, SD	.	.	.	7.3	7.2	7.4	-	7.4	7.4	7.5	-
Lennox, SD	.	.	.	7.5	7.4	7.6	-	7.5	7.4	7.6	-
Spring, Afternoon											
Jonestown, MS	7.1	5.7	8.0	7.8	7.6	8.1	0.7	7.6	6.8	8.4	0.6
Shelby, MS	7.0	5.1	8.0	8.6	7.2	10.8	1.6	9.2	8.8	9.8	2.2
El Paso, TX	.	.	.	.	.	.	-	.	.	.	-
Kermit, TX	7.3	7.2	7.5	7.2	7.1	7.3	0.1	8.2	7.5	8.6	0.9
Beresford, SD	.	.	.	8.1	7.5	8.9	-	8.1	7.8	8.5	-
Lennox, SD	.	.	.	7.7	7.5	7.8	-	8.1	7.8	8.7	-

* Not done.

TABLE A-8. MEAN DISSOLVED OXYGEN VALUES (mg/L) AND DIFFERENCES FROM INFLUENT TO LAGOONS IN NONAERATED SYSTEMS

Season/Site	Influent			Pond I Effluent				Pond II Effluent			Overall
		Range			Range				Range		
	Mean	Min	Max	Mean	Min	Max	Δ DO	Mean	Min	Max	Δ DO
Summer, Morning											
Jonestown, MS	1.2	0.4	1.8	6.2	2.5	8.9	5.0	4.6	2.4	6.6	3.4
Shelby, MS	0.5	0.0	1.0	9.8	7.0	13.2	9.4	8.1	6.3	8.8	7.6
El Paso, TX	0.1	0.0	0.1	1.5	1.0	2.0	1.4	2.5	2.1	3.0	2.4
Kermit, TX	0.5	0.2	1.0	4.7	0.2	13.0	4.2	1.2	0.3	2.2	0.8
Beresford, SD	2.9	1.8	4.3	8.0	4.0	16.2	5.1	3.3	0.9	6.1	0.4
Lennox, SD	0.5	0.0	1.9	2.0	0.5	3.3	1.5	3.9	2.0	5.5	3.4
Winter, Morning											
Jonestown, MS	1.9	1.3	2.5	7.1	5.3	8.8	5.2	7.3	5.6	8.9	5.4
Shelby, MS	1.5	0.2	2.9	8.8	6.0	11.1	7.2	8.3	6.1	9.8	6.7
El Paso, TX	0.0	0.0	0.0	2.7	2.3	3.1	2.7	2.6	2.5	2.8	2.6
Kermit, TX	0.3	0.2	0.3	0.3	0.2	0.3	0.0	4.3	1.5	7.0	4.0
Beresford, SD	4.3	3.5	5.0	0.1	0.5	0.1	-4.2	0.1	0.1	0.1	-4.2
Lennox, SD	2.5	2.2	2.8	0.1	0.1	0.2	-2.4	0.1	0.1	0.1	-2.5
Spring, Morning											
Jonestown, MS	1.4	1.0	1.6	10.1	5.4	16.4	8.7	10.0	7.0	15.6	8.6
Shelby, MS	2.3	1.6	2.8	12.2	7.2	20.0	9.8	12.2	8.0	18.6	9.8
El Paso, TX	0.1	0.1	0.2	2.2	1.8	2.9	2.1	2.2	1.5	3.0	2.1
Kermit, TX	0.4	0.3	0.4	3.6	0.4	7.0	3.3	11.7	7.0	14.0	11.3
Beresford, SD	3.3	1.6	4.3	9.6	1.2	24.9	6.3	6.6	3.5	11.9	3.3
Lennox, SD	1.5	0.3	2.2	0.2	0.1	0.6	-1.2	8.1	1.8	20.0	6.6
Summer, Afternoon											
Jonestown, MS	2.0	1.0	2.7	10.0	6.0	12.6	8.0	9.3	6.0	13.1	7.3
Shelby, MS	1.2	0.0	1.9	13.9	10.2	17.2	12.7	13.2	8.0	17.6	12.1
El Paso, TX	.*	.	.	1.6	1.2	2.0	-	2.5	2.0	3.1	-
Kermit, TX	0.4	0.2	0.7	2.3	0.3	7.0	1.9	2.8	1.0	4.5	2.4
Beresford, SD	.	.	.	13.7	7.2	28.7	-	4.4	1.3	6.9	-
Lennox, SD	.	.	.	7.0	1.3	14.2	-	5.6	2.8	7.9	-
Winter, Afternoon											
Jonestown, MS	1.0	1.0	1.0	7.5	5.4	9.5	6.5	7.6	5.7	9.4	6.6
Shelby, MS	0.9	0.0	1.7	7.0	5.2	9.3	6.9	10.2	6.7	15.6	9.3
El Paso, TX	.	.	.	.	.	.	-	.	.	.	-
Kermit, TX	0.4	0.3	0.5	0.3	0.2	0.4	-0.1	6.3	3.5	9.0	5.9
Beresford, SD	.	.	.	0.1	0.1	0.1	-	0.1	0.1	0.1	-
Lennox, SD	.	.	.	0.1	0.1	0.1	-	0.1	0.1	0.1	-
Spring, Afternoon											
Jonestown, MS	1.1	1.0	1.2	14.3	5.7	20.0	13.2	13.3	5.5	19.0	12.2
Shelby, MS	1.7	0.1	2.9	12.0	7.2	20.0	10.2	9.8	7.2	13.6	8.0
El Paso, TX	.	.	.	.	.	.	-	.	.	.	-
Kermit, TX	0.3	0.2	0.3	2.6	0.4	4.8	2.3	11.3	5.8	15.0	11.0
Beresford, SD	.	.	.	11.6	0.8	29.0	-	7.6	4.3	13.0	-
Lennox, SD	.	.	.	0.2	0.1	0.5	-	9.4	0.9	22.8	-

* Not done.

TABLE A-9. GEOMETRIC MEAN VIRUS CONCENTRATION (PFU/L), CONCENTRATION RANGES AND PERCENT VIRUS REDUCTION, AS DETERMINED ON BGM CELL LINES, NONAERATED SITES

Season/Site	Influent			Pond I Effluent			Percent Removal	Pond II Effluent			Overall Percent Removal
	Mean	Range Min	Range Max	Mean	Range Min	Range Max		Mean	Range Min	Range Max	
Summer											
Jonestown, MS	514	33	2,133	4.8	0.2	403	99.87	0.2	0.1	0.3	99.97
Shelby, MS	791	206	3,979	12.7	6.2	24.4	98.40	0.8	0.1	7.7	99.90
El Paso, TX	348	71	5,756	4.6	1.4	17.4	98.67	0.6	0.5	0.8	99.83
Kermit, TX	180	21	785	7.5	0.3	113.0	95.86	1.3	0.2	6.4	99.28
Beresford, SD	94	14	1,437	0.6	0.0	2.0	99.41	0.5	0.1	1.4	99.45
Lennox, SD	162	72	445	0.5	0.2	2.1	99.71	0.3	0.1	2.1	99.80
Winter											
Jonestown, MS	32	20	63	1.1	0.7	1.9	96.75	0.3	0.2	0.7	99.01
Shelby, MS	52	22	139	2.6	0.2	18.0	95.02	0.7	0.2	4.8	98.62
El Paso, TX	87	63	160	2.6	0.5	15.6	96.96	1.0	0.6	2.4	98.85
Kermit, TX	148	71	285	33.6	17.3	60.6	77.36	9.1	7.7	12.4	93.83
Beresford, SD	45	45	45	15.2	15.2	15.2	68.50	8.2	8.2	8.2	95.06
Lennox, SD	216	59	605	11.3	4.5	22.4	94.77	10.5	5.0	18.9	95.12
Spring											
Jonestown, MS	83	32	217	0.5	0.4	0.6	99.41	0.3	0.3	0.4	99.63
Shelby, MS	53	24	101	0.3	0.1	0.6	99.52	0.2	0.1	0.5	99.54
El Paso, TX	74	38	137	14.3	12.5	16.4	80.59	1.1	0.3	3.4	98.51
Kermit, TX	52	6	216	11.7	1.0	117	77.49	5.6	0.9	15.5	89.27
Beresford, SD	50	28	96	2.0	0.2	21.6	96.03	0.4	0.2	1.0	99.27
Lennox, SD	17	3	70	1.2	0.5	4.3	92.48	0.3	0.2	0.8	98.21

TABLE A-10. GEOMETRIC MEAN VIRUS CONCENTRATION (PFU/L), CONCENTRATION RANGES AND PERCENT VIRUS REDUCTION AS DETERMINED ON HELA CELL LINES, NONAERATED SITES

Season/Site	Influent			Pond I Effluent			Percent Removal	Pond II Effluent			Overall Percent Removal
	Mean	Range Min	Range Max	Mean	Range Min	Range Max		Mean	Range Min	Range Max	
Summer											
Jonestown, MS	106	27	3,319	1.9	0.2	23.9	98.18	0.2	0.1	0.3	99.83
Shelby, MS	41	4	300	1.7	0.3	5.2	95.85	0.5	0.1	6.2	98.78
El Paso, TX	64	43	94	2.8	1.7	3.9	95.63	0.4	0.0	0.8	99.41
Kermit, TX	13	5	52	7.2	1.5	22.7	44.02	0.8	0.2	2.2	93.50
Beresford, SD	30	19	40	0.4	0.3	0.4	98.83	0.4	0.1	1.6	98.84
Lennox, SD	38	19	112	0.8	0.2	8.3	97.90	0.3	0.1	5.3	99.15
Winter											
Jonestown, MS	17	14	28	0.9	0.2	2.9	94.73	0.2	0.2	0.3	98.68
Shelby, MS	52	24	78	1.4	0.3	27.7	97.23	0.2	0.1	0.2	99.63
El Paso, TX	42	27	66	0.5	0.4	0.6	98.76	0.8	0.4	2.1	98.09
Kermit, TX	55	30	102	4.4	1.0	9.3	92.00	0.7	0.3	4.6	98.63
Beresford, SD	26	13	56	7.9	2.6	15.4	70.23	0.7	0.1	2.7	97.19
Lennox, SD	266	59	1,208	2.9	1.4	7.2	98.92	1.5	0.2	8.5	99.42
Spring											
Jonestown, MS	-	-	-	0.4	0.4	0.4	-	0.2	0.2	0.2	-
Shelby, MS	106	106	106	0.3	0.3	0.3	99.72	0.3	0.1	0.6	99.71
El Paso, TX	99	50	195	43.5	35.5	53.2	56.14	1.7	0.4	7.8	98.24
Kermit, TX	17	12	23	0.9	0.3	2.3	94.76	1.8	1.4	2.2	89.38
Beresford, SD	56	53	59	1.5	0.7	3.3	97.30	1.0	0.3	3.5	98.24
Lennox, SD	35	5	231	1.8	1	3.1	94.83	0.6	0.3	1.5	98.16

TABLE A-11. GEOMETRIC MEAN VIRUS CONCENTRATIONS, (PFU/L), CONCENTRATION RANGES AND PERCENT VIRUS REDUCTION AS DETERMINED ON RD CELL LINES, NONAERATED SITES

Season/Site	Influent			Pond I Effluent			Percent Removal	Pond II Effluent			Overall Percent Removal
		Range			Range				Range		
	Mean	Min	Max	Mean	Min	Max		Mean	Min	Max	
Summer											
Jonestown, MS	231	62	1,015	0.8	0.2	2.8	99.67	0.2	0.1	0.3	99.92
Shelby, MS	94	76	116	1.2	0.1	4.2	98.73	0.4	0.1	1.3	99.62
El Paso, TX	336	95	1,848	2.1	1.1	3.2	99.36	0.3	0.3	0.5	99.90
Kermit, TX	197	146	266	88.6	66.2	119.0	55.02	0.6	0.2	2.9	99.71
Beresford, SD	65	48	150	0.4	0.1	1.1	99.41	0.2	0.1	0.3	99.69
Lennox, SD	270	36	1,791	1.5	0.2	18.2	99.44	0.3	0.1	1.1	99.90
Winter											
Jonestown, MS	16	5	48	0.9	0.7	1.1	94.08	0.5	0.2	1.0	96.73
Shelby, MS	68	19	129	2.3	0.5	18.7	96.67	0.2	0.2	0.3	99.68
El Paso, TX	45	16	117	4.1	0.9	29.3	90.77	1.3	0.6	2.1	97.03
Kermit, TX	92	27	318	41.1	33.6	60.6	55.20	16.4	6.9	43.4	82.09
Beresford, SD	.	.	.	.	.	.	–	.	.	.	–
Lennox, SD	106	26	682	12.7	10.1	16.5	87.97	1.8	0.2	6.0	98.30
Spring											
Jonestown, MS	307	387	307	0.7	0.7	0.7	99.79	0.2	0.2	0.2	99.93
Shelby, MS	139	139	139	0.3	0.3	0.3	99.82	0.3	0.2	0.3	99.81
El Paso, TX	46	17	112	2.8	0.8	7.6	93.96	0.9	0.3	3.7	98.05
Kermit, TX	28	21	34	16.0	10.3	22.7	42.66	1.6	0.8	2.7	94.42
Beresford, SD	32	26	39	1.0	0.6	1.7	96.69	0.3	0.1	0.5	99.20
Lennox, SD	29	28	31	0.2	0.2	0.2	99.38	0.2	0.1	0.2	99.36

APPENDIX B

LIST OF CONTRACTORS

Local area contractors who participated in on-site work and nonvirus laboratory determinations are listed in the table. Contractor addresses and the site(s) at which work was performed are included.

TABLE B-1. LIST OF CONTRACTORS

Contractor Name	Address	Sites Studied
Enviro-Labs, Inc.	603 Gillespie St. Starkville, MS 39759	Jonestown, MS Shelby, MS
El Paso Chem-Tec Laboratories	P.O. Box 1565 El Paso, TX 79948	El Paso, TX (Northeast Ponds)
Martin Water Laboratories, Inc.	P.O. Box 1468 Monahans, TX 79756	Kermit, TX
City of Sioux Falls Water Reclamation Dept.	501 East Olive Sioux Falls, SD 57104	Beresford, SD Lennox, SD
Muskegon County Wastewater Management System	Muskegon, MI 49443	Muskegon, MI
Industrial Chemical Laboratories, Inc.	2600 W. 29th Ave. Denver, CO 80211	Castle Rock, CO

GLOSSARY

Aerated lagoon: A pond designed for either partial or complete mechanical aeration. If partial, as in this study, both sludge deposition and anaerobic zones occur.

Analysis of variance: A statistical technique that achieves resolution of the effects of various factors and interactions between factors in a set of data.

Bentonite: A clay mineral of the montmorillonite group.

Enteric virus: Those viruses whose predominant site of replication is in cells lining the gut. Present in wastewater and sewage-polluted waters.

Facultative lagoon: A pond having an aerobic zone near the surface, with a gradient to anaerobic conditions near the bottom. Oxygenation occurs by wind action, diffusion and algal activity.

Holding pond: A pond designed primarily for storage rather than primarily for treatment. It is less likely to meet such treatment pond criteria as shallow depth and controlled loading, and residence time may be more variable.

Inactivation (of virus): Any change in a virus particle that renders it incapable of forming a plaque or producing infection with conditions tested. Virus may become unrecoverable through actual inactivation or irreversible adsorption to solid materials.

Indigenous virus: Virus naturally present, as opposed to virus intentionally added for investigational purposes.

Langley: A unit of solar radiation, equal to 1 cal/cm^2.

Mechanical aeration: Use of artificial mixing or other devices to accelerate the transfer of atmospheric oxygen into water.

Monolayer (cell culture): A sheet of cells, one cell in thickness, lying over and adhering to an interior surface within a culture vessel or dish.

Plaque assay: A method of enumerating viable virus particles from a suspension by counting zones of virus multiplication (plaques) on a cell monolayer.

Plaque-forming unit: A virus particle or group of particles that forms a plaque in the plaque assay method. Plaque-forming units are those virus particles that are viable as defined by the conditions of the test.

Residence time: The length of time required for an average particle of water to travel from the inlet to the outflow of a pond or pond system. Actual residence time often varies greatly due to short-circuiting, wind and other factors.

Seepage: Downward travel of water from a pond toward groundwater. Seepage is usually minimized by the use of clay or other relatively impervious material to line the ponds.